EVALUATION OF RECREATIONAL SURFACE WATER QUALITY ACCORDING TO WATER QUALITY INDEX AND QUANTITATIVE MICROBIAL RISK ASSESSMENT OF NOROVIRUSES AND SARS-COV-2

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การประเมินคุณภาพน้ำผิวดินเพื่อการสันทนาการตามดัชนีคุณภาพน้ำและการประเมินความเสี่ยงจุลินทรีย์เชิงปริมาณของโนโรไวรัสและซาร์โควีทู

น.ส.ปาริชาติ สร้อยสูงเนิน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	EVALUATION OF RECREATIONAL SURFACE WATER QUALITY ACCORDING TO WATER QUALITY INDEX AND QUANTITATIVE MICROBIAL RISK ASSESSMENT OF NOROVIRUSES AND SARS-COV-2
By	Miss Parichart Soisoongnern
Field of Study	Industrial Toxicology and Risk Assessment
Thesis Advisor	Dr. JATUWAT SANGSANONT

Accepted by the FACULTY OF SCIENCE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

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ปาริชาติ สร้อยสูงเนิน : การประเมินคุณภาพน้ำผิวดินเพื่อการสันทนาการตามดัชนีคุณภาพน้ำและการประเมินความเสี่ยงจุลินทรีย์เชิงปริมาณของโนโรไวรัสและซาร์โควิทู. (EVALUATION OF RECREATIONAL SURFACE WATER QUALITY ACCORDING TO WATER QUALITY INDEX AND QUANTITATIVE MICROBIAL RISK ASSESSMENT OF NOROVIRUSES AND SARS-COV-2) อ.ที่ปรึกษาหลัก : อ. ดร.จตุวัฒน์ แสงสานนท์

การระบาดของไวรัสจากน้ำเป็นสื่อเป็นปัญหาสำคัญทั่วโลกซึ่งอาจติดต่อผ่านทางการกลืนกินโดยไม่ตั้งใจจากกิจกรรมทางน้ำโดยปัจจุบันใช้ดัชนีคุณภาพน้ำ (Water Quality Index; WQI) เป็นเครื่องมือสำหรับการประเมินคุณภาพน้ำสำหรับกิจกรรมที่เกี่ยวข้อง อย่างไรก็ตามความเหมาะสมของกิจกรรมทางน้ำจากการประเมินความเสี่ยงที่เกี่ยวข้องกับเชื้อโรคทางน้ำยังคงเป็นที่น่าสงสัย การศึกษานี้มีวัตถุประสงค์เพื่อระบุข้อจำกัด ช่องว่างที่อาจเกิดขึ้นของ WQI และบริบทของความเสี่ยงจากเชื้อโรคในน้ำ โดยการสำรวจความชุกของไวรัสในน้ำผิวดิน การประเมินคุณภาพน้ำด้วย WQI การประเมินความเสี่ยงจุลินทรีย์เชิงปริมาณ (Quantitative Microbial Risk Assessment; QMRA) โดยใช้เชื้อโนโรไวรัสเป็นตัวแทนไวรัสประจำถิ่นและเชื้อซาร์โควิทูเป็นตัวแทนไวรัสสายพันธุ์ใหม่รวมถึงการทดสอบความสัมพันธ์ระหว่าง WQI กับ QMRA ทั้งภาพรวมตลอดการศึกษา (9 เดือน) ฤดูแล้ง และฤดูฝน ในคลองมหาสวัสดิ์ จังหวัดนครปฐม ประเทศไทย

ผลการศึกษาพบว่าตัวอย่างน้ำผิวดิน (81 ตัวอย่าง) มีความชุกของเชื้อโนโรไวรัส (ร้อยละ 34) และเชื้อซาร์โควิทู (ร้อยละ 9.9) ส่วนผลการประเมินผลคุณภาพน้ำในคลองมหาสวัสดิ์อยู่ในระดับพอใช้ (แหล่งน้ำประเภทที่ 3) ทั้งภาพรวม ฤดูแล้ง และฤดูฝนซึ่งมีความเหมาะสมต่อการนำไปใช้ประโยชน์ทางการเกษตรและการคมนาคม ในขณะที่พิจารณาคุณภาพน้ำในแต่ละเดือนพบว่าเดือนพฤษภาคมมีคุณภาพน้ำอยู่ในระดับ “ดี” ซึ่งอาจเหมาะสมต่อกิจกรรมสันทนาการทางน้ำ อย่างไรก็ตาม ผลการประเมินความเสี่ยงจุลินทรีย์เชิงปริมาณของเชื้อโนโรไวรัสและเชื้อซาร์โควิทูมีความน่าจะเป็นที่จะเจ็บป่วยจากเชื้อโนโรไวรัสในระหว่างการว่ายน้ำ การเดินทางทางเรือ และการกินผักกาดหอมที่ปลูกด้วยน้ำจากคลองมหาสวัสดิ์มากกว่า 36 ครั้งต่อการรับสัมผัส 1000 ครั้งซึ่งเกินเกณฑ์มาตรฐานที่ยอมรับได้ (≥0.036) ส่วนความเสี่ยงของเชื้อซาร์โควิทูมีความน่าจะเป็นที่จะเจ็บป่วยค่อนข้างต่ำในการเดินทางทางเรือ และการกินผักกาดหอมในขณะที่โอกาสเจ็บป่วยจากเชื้อซาร์โควิทูจากการว่ายน้ำเกินค่ามาตรฐานที่กำหนดหรือมากกว่า 1 ครั้งต่อการรับสัมผัส 1000 ครั้ง ข้อค้นพบเหล่านี้ชี้ให้เห็นว่า การใช้ WQI เพียงอย่างเดียวในการประเมินคุณภาพน้ำอาจไม่ครอบคลุมความเหมาะสมสำหรับกิจกรรมทางน้ำที่เฉพาะเจาะจงได้ ดังนั้นการใช้ QMRA ร่วมกับ WQI ในการประเมินคุณภาพน้ำจะสามารถวิเคราะห์ผลเชิงลึกได้มากกว่าโดยเฉพาะอย่างยิ่งเมื่อพิจารณาความเสี่ยงจากการปนเปื้อนของเชื้อโรค

สาขาวิชา	พิษวิทยาอุตสาหกรรมและการประเมินความเสี่ยง	ลายมือชื่อนิสิต ....
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Outbreak of waterborne virus are the major concern worldwide. The Water Quality Index (WQI) is a tool used to assess water quality for related activities. However, its suitability for assessing risks associated with waterborne pathogens remains questionable. This study aims to identify potential limitations and gaps in the WQI, especially in the context of risks from waterborne pathogens. The WQI of Maha Sawat Canal (MSC), Thailand was evaluated during both wet and dry seasons. The results were then integrated with the quantitative microbial risk assessment (QMRA) methodology for norovirus GI (NoV GI) and SARS-CoV-2. The results have shown that NoV GI (34%) and SARS-CoV-2 (9.9%) were detected in 81 water samples. The WQI analysis categorized MSC's water quality as a 'fair' level of overall nine-month event to study including wet and dry seasons, suggesting its suitability for agricultural and transportation. While considering each month, water quality was at a "good" level in May, which might relate to the first month of the wet season. However, the probability of contracting an illness from NoV GI during swimming (0.148), boat transportation (0.126) also consumed vegetables (Lettuce) from the MSC agriculture irrigation (0.225,0.229, 0.022) exceeded the acceptable benchmark of gastroenteritis illness (GI) for NoV (0.036). The risk of SARS-CoV-2 remains relatively lower in boat activity and consumed vegetables (Lettuce) from the MSC agriculture irrigation (≥0.001) while the illness from SARS-CoV-2 during swimming (0.01, 02, 0.01) exceeds the set of benchmarks for SARS-CoV-2 (0.001). These findings suggest that the WQI alone may not provide a comprehensive assessment of the suitability of water for specific activities. Thus, incorporating QMRA into the water quality evaluation can provide a more in-depth analysis, particularly when considering risks from specific pathogen contamination.

Field of Study:	Industrial Toxicology and Risk Assessment	Student's Signature ....
Academic Year:	2023	Advisor's Signature ....

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Parichart Soisoongnern

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Chapter 1
Introduction

1.1 Background and rational

Surface water contaminated with pathogens can be sources of water-borne diseases such as acute diarrhea, and gastroenteritis. The predominant cause of sporadic and epidemic gastroenteritis accounts for greater than 90% of viral gastroenteritis (Atmar et al., 2014). The global health burden of pathogenic viruses, notably norovirus, a leading cause of water borne virus diseases worldwide outbreak, is of significant concern (Gibson, 2014). The United States alone, norovirus outbreaks result in approximately 685 million cases annually (Prevention, 2023). The Ministry of Public Health's Health Data Center in Thailand reported a substantial number of viral gastroenteritis cases between 2019 and 2021. There were 20,314 cases from rotavirus, 18,323 cases from noroviruses, and 3,577 cases from adenoviruses. In addition to these endemic waterborne pathogens, emergent viruses like SARS-CoV-2 demand increased attention, especially during pandemics. The SARS-CoV-2 or COVID-19, has globally caused 617,597,680 confirmed cases with 6,532,705 deaths. In Thailand, there were 4,682,132 COVID-19 confirmed cases of with 32,771 deaths. Although the COVID-19 mainly transmitted via respiratory system and personal contact, the previous studies reported the existence of SARS-CoV-2 in the wastewater system (Sangsanont et al., 2021; Tyagi et al., 2021). In addition to these endemic waterborne pathogens, emergent viruses like SARS-CoV-2 demand increased attention, especially during pandemics. Therefore, it is crucial to continuously assess and monitor the prevalence of such viruses in the environment, especially within the water cycle.

The WQI serves as a tool to evaluate surface water quality and determined its suitability for water-related activities (Lachhab, 2019). The WQI indices consist of the physical, chemical, and biological standards that can be selected for evaluation. Commonly assessed parameters in many countries include Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Ammonia (NH_3-N), Total Coliform Bacteria (TCB), and Fecal Coliform Bacteria (FCB). The WQI offers several advantages, such as gauging basic surface water quality and classifying its status for the use of consumption, recreation, agriculture, and transportation. Microbial indicators such as TCB and FCB were used worldwide (Lumb et al., 2011) for indicating the waterborne pathogen contamination, while certain microbial pathogens present in surface water, especially viruses, protozoa, and other pathogens, are not measured by the WQI. Therefore, the indicators may not adequately represent the risk to human health by these pathogens.

QMRA is a globally used tool to evaluate risks from pathogenic microbes (Organization, 2016). It has been effectively applied in countries like South Africa, Singapore, and Norway to assess risks in surface water (Petterson et al., 2016; Van Abel et al., 2017; Vergara et al., 2016). Many nations have implemented QMRA to establish pathogen benchmarks for drinking water and to assess risks in recreational activities involving accidental ingestion of polluted water as well as implemented water safety plans (Organization, 2016; Smeets, 2019). QMRA has four key steps to apply, including hazard identification, exposure assessment, dose-response assessment, and risk characterization (Haas et al., 2014; Van Abel et al., 2017). Therefore, the probability of infection in terms of accidental ingestion during transportation by boating along the MSC should be assessed by QMRA. While WQI provides a general overview of water quality, QMRA offers deeper insights into the risks associated with waterborne pathogens. However, studies exploring the integration of QMRA with WQI to evaluate water usage suitability are still limited, highlighting a crucial area for further research.

The current study evaluated the water quality in the MSC, a tributary of the Tha Chin River, focusing on the risks posed by norovirus and SARS-CoV-2 contamination. This evaluation utilizes both the WQI and QMRA methodologies. The MSC, located in a suburban area of Bangkok, is integral to various activities such as agriculture, travel, and community living. Spanning 28 kilometers, it connects several districts and flows into the Tha Chin River. The Pollution Control Department (PCD) monitors the canal's water quality using national standards and the WQI method. The research aims to investigate the prevalence of norovirus and SARS-CoV-2 throughout different seasons and explore the relationship between WQI and QMRA in the MSC, providing insights into water quality and health risks associated with these waterborne pathogens.

1.2 Research objectives

1 .2. 1 To investigate the prevalence of norovirus and SARS-CoV-2 in the Maha Sawat canal and Tha Chin River, Nakhon Pathom, Thailand

1.2.2 To assess the suitability of water uses in Maha Sawat canal utilizing WQI and QMRA for noroviruses and SARS-CoV-2.

1.2.3 To investigate the potential relationship between WQI and quantitative viral risk associated with water activities in Maha Sawat canal.

1.3 Research questions

With the rise of emerging diseases and frequent virus outbreaks, there is growing concern about viral presence in surface water. The Water Quality Index

(WQI) alone may not fully capture the extent of viral contamination or the health risks from exposure to such water.

1.4 Research hypothesis

1.4.1 Although surface water quality status assesses by WQI indicates that surface water sources can be recreational, such as swimming or boating, the emerging and endemic virus contamination were still detected. The human health risk for waterborne pathogen contamination from water related activities is higher than guideline.

1.4.2 During the COVID-19 epidemic, surface water quality assessing by WQI did not represent the spread of SARS-CoV-2 in the surface water

1.5 Scope of the study

1.5.1 The water sampling of the surface water from the Maha Sawat canal and Tha Chin River at Nakhon Pathom during December 2021 to August 2022

1.5.2 The water quality index was calculated by using the data from Thai Pollution Control Department (PCD), Climate Change and Environmental Research Center, and Mahidol University. Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Fecal Coliform Bacteria (FCB), and Total Coliform Bacteria (TCB) were obtained from Thai Climate Change and Environmental Research Center, and Mahidol University in the same sampling periods. NH_3-N data was obtained from PCD because of inadequate NH_3-N field data for WQI calculation. PCD's surface water monitoring data at Maha Sawat Canal (MSC) station and Tha Chin River (TC13) station between 2 seasons, the dry season (December,2021 to April 2022) and the wet season (May to August, 2022).

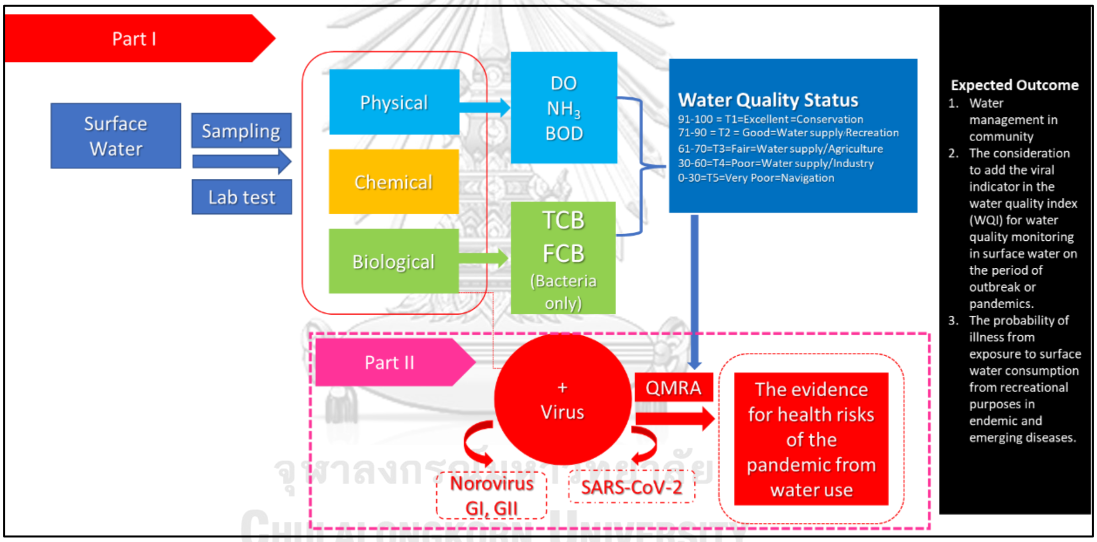
1.5.3 The concentration of norovirus GI, GII and SARS-CoV-2 was analyzed by the RT-qPCR assay.

1.5.4 QMRA was calculated dose respond by using the data from the study and using references dose from previous researches.

1.5.5 The risk scenario was used the surface water quality status (Type 1 – 5) and focus on the recreation water by ingestion during swimming, boat transportation, and vegetable consumption using irrigated water from the canal.

1.6 Conceptual Framework

Evaluation of surface water quality according to WQI provided by the PCD that used water quality data in the same sampling site from Climate Change and Environmental Research Center, and Mahidol University, only NH_3-N was used a surface water quality data from the PCD. Moreover, water quality status that assessed by WQI were using to defined scenario to hazard identification of QMRA step. After that, the QMRA of noroviruses and SARS-COV-2 was used to assess the risk of ingesting surface water during recreation by swimming, consuming vegetables, and boating transportation. Finally, the evidence of health risks of the study was serving the probability of illness related water activities form the evaluation of WQI and QMRA. Furthermore, the expected outcome that a study result was serve the consideration to add the viral indicator to the WQI on the period of the outbreak and the pandemics. The framework is summarized briefly in Figure 1.



Water Quality Status
91-100 = T1=Excellent =Conservation
71-90 = T2 = Good=Water supply/Recreation
61-70=T3=Fair=Water supply/Agriculture
30-60=T4=Poor=Water supply/Industry
0-30=T5=Very Poor=Navigation

Expected Outcome
1. Water management in community
2. The consideration to add the viral indicator in the water quality index (WQI) for water quality monitoring in surface water on the period of outbreak or pandemics.
3. The probability of illness from exposure to surface water consumption from recreational purposes in endemic and emerging diseases.

Figure 1 Conceptual Framework

Chapter 2
Literature Review

2.1 Surface water

The world’s surface is covered by water, where 96.5 percent is the oceans and only 0.3 percent is surface water resources. The surface water is the water above the ground including rivers, streams, lakes, reservoirs, wetland, creeks and swamps. This surface water is significant to use for human activities such as irrigation, agriculture, fishery, domestic and also drinking. Additionally, fecal pollution of surface water originates from a range of human-associated sources and affects both rural and urban locations (Schwab et al., 1995). All nations are affected by endemic and epidemic diseases brought on by an unsafe water supply. In both developed and developing nations, there are still water-borne disease outbreaks, resulting in death, illness, and financial hardship for individuals and communities. It is anticipated that improvements in excreta disposal and personal hygiene, along with strategies to improve water quality, will result in significant improvements in population health (Davison et al., 2005).

2.2 Recreation water

World Health Organization define the recreation water is the use of coastal, estuarine and freshwater recreational environments that provides numerous advantages for one's health and wellbeing, including rest, relaxation, physical activity, engagement in religious and cultural activities, and enjoyment of the arts (Organization, 2021). There are global concerns on health affected from water bodies, water activity, and water recreation in fresh water and coastal water. World Health Organization has launch a quantitative microbiological risk assessment application for water safety management to help an assessment of the catchment worldwide (Organization, 2016, 2021).

For the country's depth concern about water quality for recreation in Singapore, they studied especially on recreation water to protect their people from health risks posed in water where they quantified risks associated with human adenoviruses (HAdV) and noroviruses (NoV) in an urban catchment area (Vergara et al., 2016). According to the result of a recreational water study in Singapore found that norovirus (NoV) is more prevalent in water samples than adenovirus (HAdV), the QPCR testing of intestinal viruses was the primary focus of the Singapore recreational water research. In all recreation-related exposure situations, the risk of related illnesses is greater. Because norovirus is more prevalent and poses a greater risk than HAdV, norovirus is a better choice for use as a reference pathogen in Singapore's recreational waters. In addition, several studies cited a Singapore water study on the development of water biological indicators (DeFlorio-Barker et al., 2018; McBride et al., 2013).

2.3 The Water Quality Index (WQI)

2.3.1 Global water quality index

WQI is an index that offers a clear and easy way to assess the suitability of water bodies for a range of applications, including fishing, swimming, drinking, irrigation, and spawning. The Environmental Performance Index (EPI) is a comprehensive tool that includes 25 environmental performance metrics. (Lumb et al., 2011) such most country applied to assessed surface water. The WQI model was initially used in 1960 and become a well-liked instrument for assessing the quality of surface water because of its universal design and simplicity of use worldwide as in Figure 2. The WQI models consists of four stages; 1) choosing the parameters related to water quality, and 2) creating sub-indices for each parameter (3) the parameter weighting values are calculated; (4) the sub-indices are aggregated to calculate the overall water quality index.

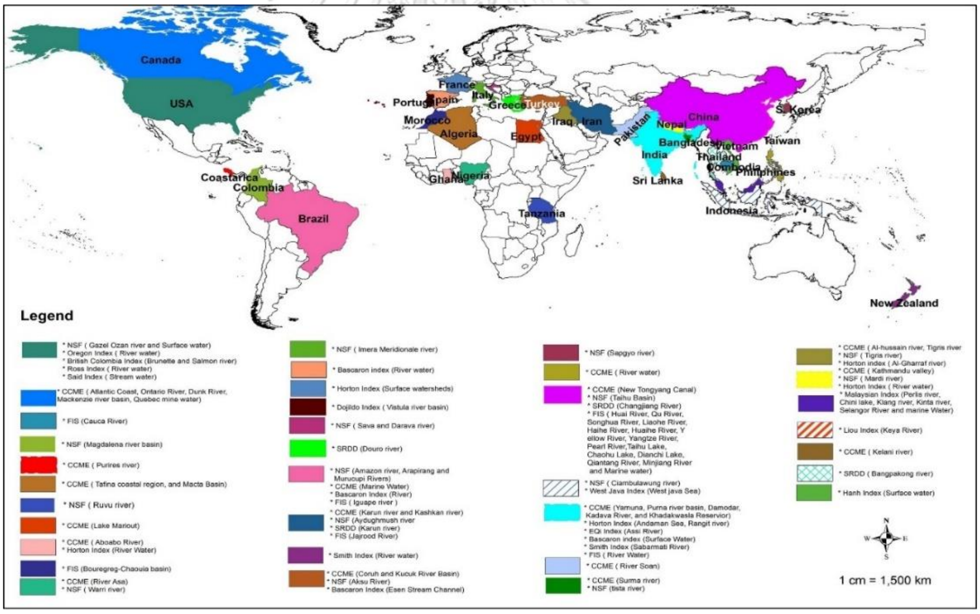


Figure 2 The WQI implementation global historical (Uddin et al., 2021).

Figure 2 shown the WQI implemented to assessed the river each country by national surface water, example USA used the National Sanitation Foundation (NSF) assessed Gazel Ozan River.

The National Sanitation Foundation Water Quality Index of British, and Columbia has regarded as a thorough and widely used index for the categorization of surface water resources according to their water quality. Nine parameters make up the index consist of dissolved oxygen (DO) saturation, pH, total solids (TS), five-day biochemical oxygen demand (BOD5), turbidity (Turb), total phosphate (TP), nitrate (NO_3^-), temperature change (T), and fecal coliform (FC). The index is based on these parameters (Noori et al., 2019). Currently, the numerical parameters of WQI depend on the water resources and functional components of each country. The assumption for calculate the score of water quality is in the equation 1.

$$\mathrm{WQI} = \sum_{i=1}^{n} W_i\ I_i \qquad \text{... (1)}$$

When WQI is the water quality index (score). W_i is the weight according to the significance of each parameter, type by (i = 1 to n). I is the grade obtained from the mean curve, where (i = 1 to n) N is the water quality used for all calculations. In additionally, the WQI was ranking score from 0 – 100 to grading water quality status such as very poor (0-30), poor (31-60), fair (61-70), good (71-90), excellent (91-100) then summarize the total score compare with indicators after that the water quality status could be indicated water quality standard, details as Table 1 (Uddin et al., 2021)

Table 1 The water quality indicators score, water quality status, and standard of surface water quality.

Water quality Indicators (score)	**Water quality status**	**Standard of surface water quality**
0 – 30	Very poor	Types 5
31 – 60	Poor	Types 4
61 – 70	Fair	Types 3
71 – 90	Good	Types 2
91 – 100	Excellent	Types 1

Regarding to the WQI development, was developed from past to present for the advantage and essential to to include more crucial factors in an index to identify specific issues with water quality; however, the WQI developer in the past was unable to incorporate the new parameters necessary for the future index application. as (Akhtar et al., 2021) summarized the WQI initial to present including first phase, second phase, and third phase that fixed system of water quality index from initially phases until 3. Due to first phase, the water quality assessment highlighted of gathering and testing of water samples in the lab. Then, second phase were defined 3 component including use of water quality standard, expert judgment, and statistical analysis by 4 keys process as selection of the parameter, formation of sub-indices, establishing weight, and aggregation of final index. Hence, third phase was a combination of first phase and second phase that obtain index value to water management 4 group related activities consist of drinking, domestic, irrigation, and industries. However, the overall 3 phase linked historical water quality assessment, WQI development, and WQI application that summarized step by step of 3 phases in Figure 3

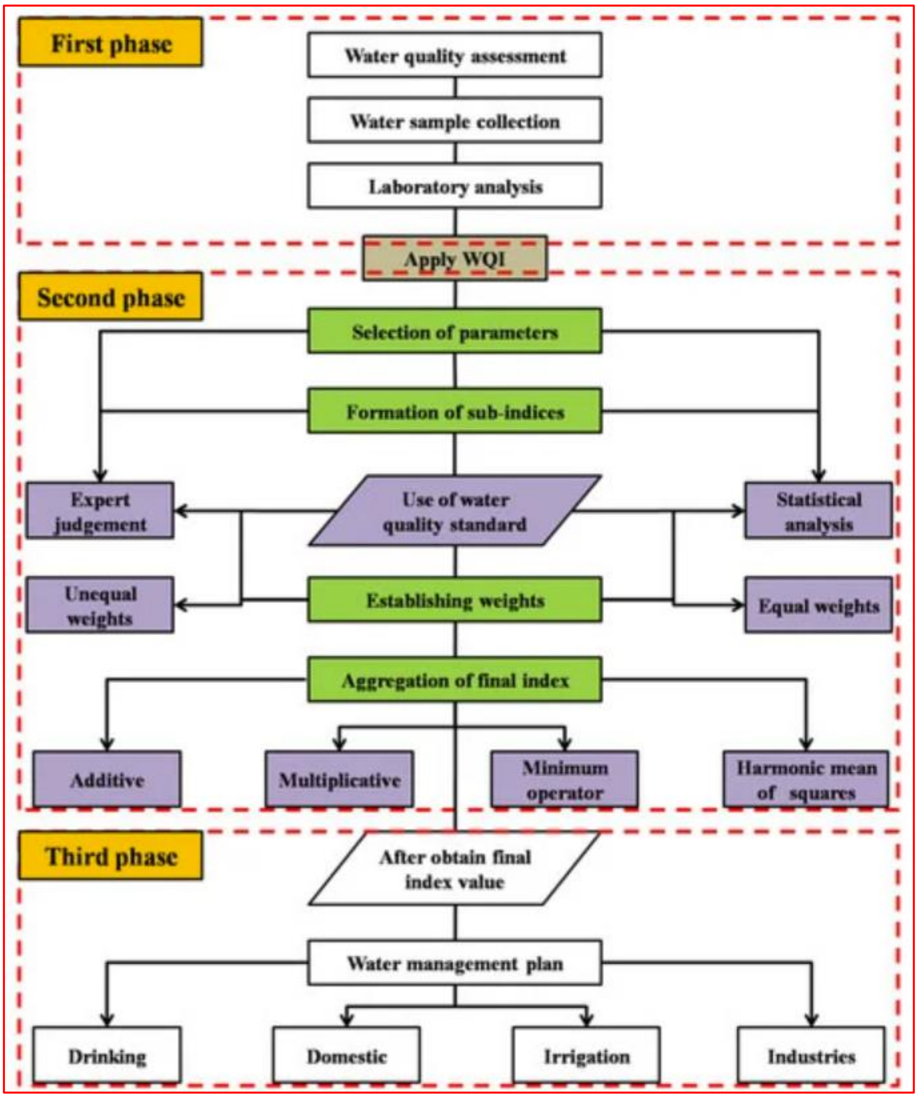


Figure 3 The significant of WQI development from initial to present (phase 1-3)

2.3.2 Thailand’s water quality index

2.3.2.1 Developing of Thailand’s WQI

Twelve criteria were used in Thailand to evaluate the quality of water: pH, Temperature, Electrical Conductivity (EC), Ammonia (NH_3-N), Dissolved Oxygen (DO), Nitrate (NO_3-N), Total Phosphorous (TP), Chemical Oxygen Demand (COD) Biochemical Oxygen Demand (BOD), Total Solid (TS), Total sSspended Solid (TSS) and Fecal Coliform Bacteria (FCB) (Sukthanapirat, 2017). In 2008, Bureau of Water Quality Management of Pollution Control Department (PCD) has recommended to used 8 parameters but some parameters was not included in surface water quality standard according to the previous study (Simachaya, 2000). At present, Thailand selects 5 parameters to assessed water quality consist of DO, BOD, TCB, FCB, and NH_3-N as well as the 4 other criteria (Department, 2010) as follows;

1) The parameter exists on the surface water quality standard.
2) The parameter uses to assess the type of surface water resources.
3) If the parameter does not exist in the surface water quality standard, then a new parameter must be used to assess the water pollution situation.
4) If the parameter does not exist in the surface water quality standard, then new parameter must give benefit on heath environmental system (Chooaksorn, 2011). Additionally, previous research on the development of WQI in Thailand studied some of the main rivers such as Chao Phraya, Bang Pakong, Chanthaburi (Bordalo et al., 2001; Lohani & Todino, 1984; Maketon et al., 2000). Finally, the WQI equation from Thailand’s pollution control department is in the equation 2

$$\mathrm{WQI}_{\text{total score}} = \frac{(\sum_{i=1}^{n} \mathrm{WQIi})}{\mathrm{N}} \qquad \text{... (2)}$$

When; WQI = parameter

N = total parameter

n = Score of water quality

Therefore, the aggregation of the WQI (score 0 – 100) and grading the water quality status as Wongaree (2019) research the PCD’s WQI application summarized that water quality indicators, water quality status, and standard of surface water quality as Table 2

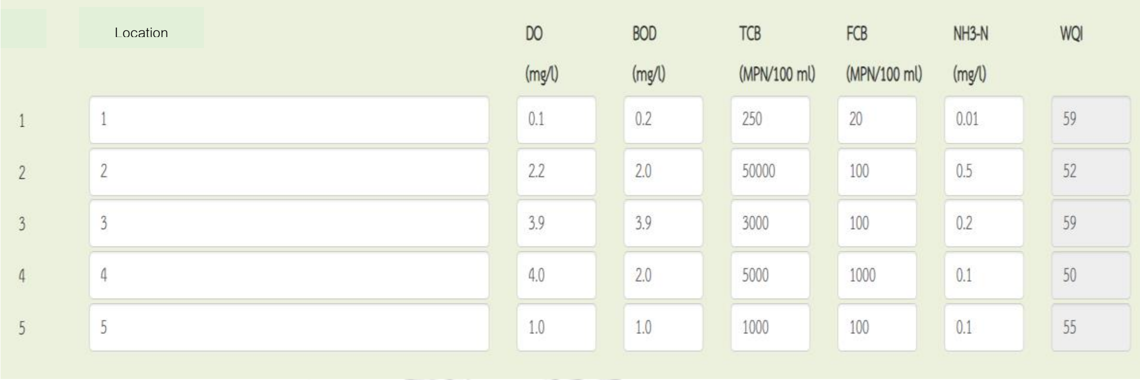
Table 2 Thai PCD’s WQI

Water quality Indicators (score)	Water quality status	Standard of surface water quality
91 – 100	Excellent	Types 1 Extra clean fresh surface water resources that are utilized for conservation do not always require a pass-through water treatment method. Instead, they simply need a standard procedure for pathogen removal and ecosystem preservation, which allows for the natural breeding of simple organisms.
71 – 90	Good	Types 2 good clean surface water resources that are used for fishing, recreation, and the conservation of aquatic life. They require pre-treatment before consumption.
61 – 70	Fair	Types 3 Medium clean fresh surface water resources used for agriculture but first go through a standard treatment procedure.
31 – 60	Poor	Types 4 Fairly clean fresh surface water supplies that are used by industry and require a specific water treatment procedure before use.
0 – 30	Very Poor	Types 5 The sources employed for navigation that do not fall within class 1-4 typically deviate from levels that are desirable or natural.

Example of the water quality index calculation by Thai-PCD water quality data on the IWIS-PCD data center (Pollution Control Department, 2018b) by defined the water quality monitoring data of the MSC as

DO = 0.1 mg/L, BOD= 0.1 mg/L, NH_3-N =0.01 mg/L, TCB=250 MPN/100 mL, FCB=20 MPN/100 mL

Then, key in to IWIS data system each parameter including the water quality value (Figure 4) or used the PCD-WQI data table (Table 3) to calculation the WQI on worksheet as shown in



	Location	DO (mg/l)	BOD (mg/l)	TCB (MPN/100 ml)	FCB (MPN/100 ml)	NH3-N (mg/l)	WQI
1	1	0.1	0.2	250	20	0.01	59
2	2	2.2	2.0	50000	100	0.5	52
3	3	3.9	3.9	3000	100	0.2	59
4	4	4.0	2.0	5000	1000	0.1	50
5	5	1.0	1.0	1000	100	0.1	55

Figure 4 The general WQI calculation on IWIS data system, Thailand

Figure 4 shown data virtualization of the general WQI calculation on IWIS data system consist of the location of monitoring station, the water quality (DO, BOD, TCB, FCB, NH_3-N) and WQI score.

The WQI calculation on the worksheet compared with the PCD-WQI criteria score had several steps. In addition, used water quality data was compared with the PCD-WQI, and then the total score of each parameter minus a different score between the Total score and the adjustment score.

Step 1; The PCD-WQI calculation 1

DO = 0.1 mg/L, BOD= 0.1 mg/L, NH_3-N =0.01 mg/L, TCB=250 MPN/100 mL, FCB=20 MPN/100 mL

Compare with the PCD-WQI data table score as Table 3.

Therefore;

$$\mathrm{WQI} = \frac{\mathrm{DO}\ (2) + \mathrm{BOD}\ (98) + \mathrm{TCB}\ (99) + \mathrm{FCB}\ (99) + \mathrm{NH3{-}N}\ (99)}{5}$$

WQI total score = 79.4 (Good)

Table 3 The PCD-WQI data table score (Polution Control Department, 2018)

DO	score	BOD	score	TCB	score	FCB	score	NH3-N	score
0.0	0	0.0	100	0	100	0	100	0.00	100
0.1	2	0.1	98	250	99	20	99	0.01	99
0.2	3	0.2	96	260	98	60	98	0.02	97
0.3	5	0.3	94	440	97	90	97	0.03	96
0.4	6	0.4	92	610	96	130	96	0.04	95
0.5	8	0.5	90	780	95	160	95	0.05	93
0.6	9	0.6	88	950	94	190	94	0.06	92
0.7	11	0.7	86	1,130	93	230	93	0.07	91
0.8	12	0.8	85	1,300	92	260	92	0.08	89
0.9	14	0.9	83	1,470	91	300	91	0.09	88
n	n	n	n	n	n	n	n	n	n

Step 2; The PCD-WQI calculation 2

Put the water quality compared with the water quality range score as the water criteria score details in Table 4

Table 4 The water quality criteria score

Parameter	**The water quality criteria score**			
	Good	**Fair**	**Poor**	**Very Poor**
	> 71	> 61	> 31	> 0
DO	> 4.0 mg/L	> 2.5 mg/L	> 2.0 mg/L	> 0.0 mg/L
BOD	< 1.5 mg/L	< 2.0 mg/L	< 4.0 mg/L	> 4.0 mg/L
TCB	< 5,000 MPN/100mL	< 20,000 MPN/100mL	> 20,000 MPN/100mL	
FCB	< 1,000 MPN/100mL	< 4,000 MPN/100mL	> 4,000 MPN/100mL	
NH_3-N	< 0.22 mg/L	< 0.50 mg/L	< 1.83 mg/L	1.83 mg/L

Therefore; summarized a total score of the calculation 1 and the calculation 2

Calculation 1

Parameter	**DO**	**BOD**	**TCB**	**FCB**	**NH_3-N**
Water quality value	0.1 mg/L	0.1 mg/L	250 MPN/100 mL	20 MPN/100 mL	0.01 mg/L
Score	2	98	99	99	99
Average score			79		
Water quality status			**Good**		

Calculation 2

Parameter	**DO**	**BOD**	**TCB**	**FCB**	**NH_3-N**
Water quality value	0.1 mg/L	0.1 mg/L	250 MPN/100 mL	20 MPN/100 mL	0.01 mg/L
Water quality criteria	Very poor	Good	Good	Good	Good
Aggregated water quality			Good		
Different level 0			0		
Total score			**79-0= 79**		

Hence, The WQI total score was 79, good water quality status suited for recreation by the water quality type 2 standard.

Regarding example of the WQI calculation above, (Wongaree, 2019) research on implementing the PCD-WQI to assess the polluted water in the local organization administrative to manage water sanitation in the community utilized by the equation below.

$$\mathrm{WQI}_{\mathrm{total\ score}} = \frac{\sum_{\mathrm{i}=1}^{\mathrm{N}} \mathrm{WQI}_{\mathrm{i}}}{\mathrm{N}} - \text{Adjustment value of the rating} * \qquad \ldots (3)$$

* The special score value is the value used to adjust the mean scores of all 5 parameters by the type of surface water source. If the water quality criteria are not different, the special score is 0. If 1 level difference, the special score is 10, 2 level difference, the special score is 15, 3 level difference, the special score is 20. Subtract the special score from the mean of the scores for all 5 parameters is the total score of that measurement point.

2.3.2.2 The implementation of Thailand's WQI

In 1995, Thailand began using WQI for the first time. The idea came from the United States of America and was to make information easy to understand for the general public.(Pollution Control Department, 2018c). Next, WQI was developed in 2010 to be statistically more compliant and is still used today. The Pollution Control Department is the main unit for designing and determining tools for collecting water quality measurement results. Both the water quality results are according to the surface water quality standards and the water quality assessment using WQI. The Office of Environment and Pollution Control region 1-16 is responsible for the main river of the country, requiring monitoring of water quality and also assessing the water quality(Ratchaburi, 2018). For example, the WQI implementation show that, according to a 2018 assessment from the Ministry of Natural Resources and Environment Pollution Control Department, surface water quality has improved and is still considered to be fair to good. Lower Chao Phraya, Lower Lamtakhong, Lower Tha Chin, Upper Phangrad, and Middle Tha Chin are the top five rivers with the worst water quality. Some low-quality water resources have declined, primarily in the estuaries of Central Thailand. Maha Sawat canals is a part of Tha Chin River that the report of the Pollution Control Department shows the results of monitoring the water quality. Tha Chin River, canals tributaries, and overlap rivers for the year 2020 found that Maha Sawat canals trend to good water quality (Pollution Control Department, 2018a) as a water quality situation between 2019 and 2021, the Tha Chin River's water quality index and the Maha Sawat canal's water quality index were both in poor condition as show in Figure 5.

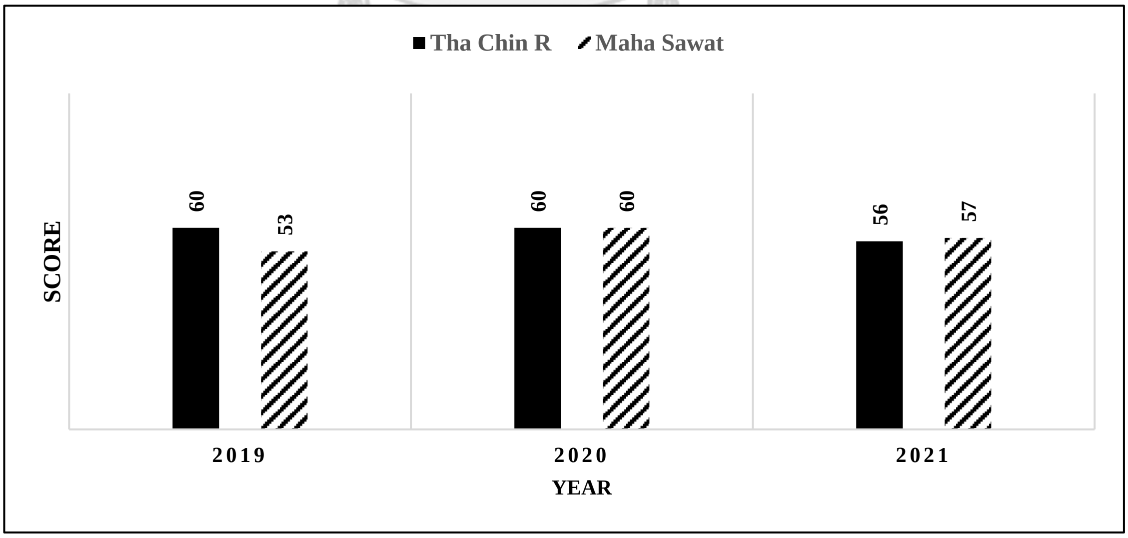


Figure 5 Surface water quality situation of The Tha Chin River and the Maha Sawat Canal in 2019-2021

Figure 5 modified the PCD-IWIS on surface water quality of the Tha Chin River (TC) and the Maha Sawat Canal (MSC) in 2019-2020.

Tha Chin river is a branch of the Chaopraya River, where it separates on the right side to the Chaopraya River at Chainat province. The Tha Chin River has 323 km length and flow through 5 provinces before goes into the Gulf of Thailand. The river has 5 tributaries; Chedi Bucha, Maha Sawat, Phasi Charoen, Damnoen Saduak, and Mahachai. The Tha Chin River were reported at poor water quality status since 1994 (Dapartment, 1994). According to the Pollution Control Department (PCD), throughout the last ten years, there has been a significant decline in the quality of water in rivers that feed into the upper Gulf of Thailand. The estuary was discovered to include bacteria as well as phosphate, phosphorus, and nitrogen fertilizer contamination. Algae develop more quickly than the ecosystem can support them because of the nutrient contamination. As a result, aquatic animals' and marine environments' access to food was significantly impacted by the quality of the water. Furthermore, it also reduces the dissolved oxygen that fish need to survive. The Pollution Control Department rated the river water quality in 2015 as "poor"(Department, 23 July 2022) from discharge of a large amount of wastewater into the river from households, industry and agriculture (Paritta, 2016). However, from an annual report the Phasi Charoen, Damnoen Saduak and Mahachai branch had good water quality while Chedi Bucha and Maha Sawat has poor water quality.

Consequence of the WQI cloud general assessed water quality especially the biological indicators as TCB and FCB might coverage virus pathogens in surface water as (Keswick et al., 1982; Lenaker et al., 2018; Marzouk et al., 1980) research a biological indicator persistence of viruses in water is higher than in bacteria, with the rise of emerging diseases and frequent virus outbreaks, there is growing concern about viral presence in surface water. The Water Quality Index (WQI) alone may not fully capture the extent of viral contamination or the health risks from exposure to such water.

This study was concerned with the emerging viruses and epidemic viruses during the COVID-19 situation, noroviruses represented endemic viruses, and SARS-CoV-2 represented epidemic viruses. The specific data of viruses and references research about the detection in surface water, method, hazard identification also quantitative microbial risk assessment by virus pathogens were described in 2.4 - 2.6.

2.4 Noroviruses

Norovirus, an RNA virus of the family *Caliciviridae*, is a human intestinal infection that significantly increases morbidity in both community and healthcare settings. The virus spread due to a number of variables, including as its long half-life in the environment, little inoculum necessary to cause infection (100 viral particles), and sustained viral shedding (Robilotti et al., 2015). The incidence of norovirus has led to, on average, 570–800 fatalities, 56,000–71,000 hospital admissions, 400,000 ER visits, 1.7–1.9 million outpatient visits, and 19–21 million illnesses annually in the United States. Elderly people are most vulnerable to mortality from a norovirus, while children under the age of five are most likely to require medical attention due to a norovirus. Year-round endemic norovirus sickness was seen, with a notable rise in December cases by 50% in the years when the pandemic strains first appeared (Hall et al., 2013). There are multiple ways that norovirus can spread, including oral and fecal-oral transmission (Figure 6). The virus can be transmission directly from person to person ; however, an indirect transmission can also occur via environment, food and water contamination (Lopman et al., 2012).

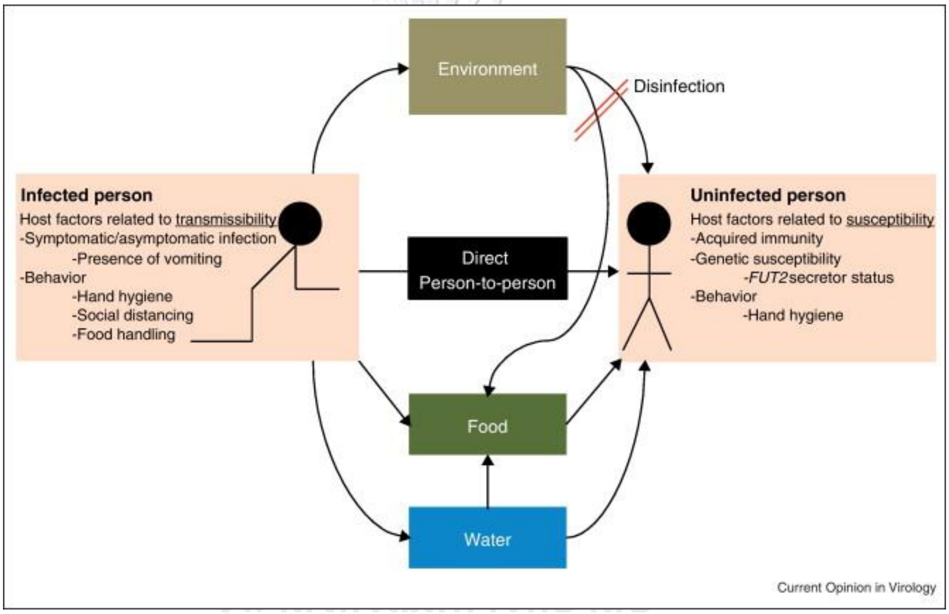


Figure 6 Route of norovirus transmission (Lopman et al., 2012)

Figure 6 presents a route of norovirus transmission in 4 ways environment, direct person-to-person, food, and water by current opinion in the virology.

The study on norovirus in Taiwan revealed that comparison between the GI group, norovirus genogroup GII was more common. The most common genotypes were GII.4 (21.2%) and GII.17 (18.2%), which matched the study's clinical conclusions. Only temperature, pH, and dissolved oxygen showed significant correlations with norovirus presence. Compared to the spring, summer, and fall, the winter had a greater Norovirus population. The results of the norovirus detection were statistically examined by looking into how they related to markers of water quality (Nagarajan et al., 2021). According to a prior study on the quantitative microbiological risk assessment of norovirus, the frequency of detection of the virus was 60.4% in crops and 37.5% in irrigation waters, respectively. Distribution characteristics of norovirus genogroups were discovered in irrigation fluids and green crops. In order to lower the risk to the public's health from consuming these infected foods, it was imperative to establish surveillance programs to identify and ensure the virological quality of food along the entire manufacturing chain (Alegbeleye & Sant'Ana, 2021).

2.5 SARS-CoV-2

SARS-CoV-2 is in the (Family) *Coronaviridae* is with a single strand of RNA, which is very stable at 4°C for 14 days. It is a new member in the family *Betacoronavirus*, like SARS-CoV and MERS-CoV (Chin et al., 2020). The transmission of SARS-CoV-2 is via aerosols or droplets. It was discovered that feces shedding persisted for at least 21 days after the symptoms disappeared. Nevertheless, there is currently no proof that SARS-CoV-2 is contagious in wastewater. Global surface water bodies and untreated sewage have both been found to contain virus RNA (Mohapatra et al., 2021). In addition, SARS-CoV-2 was transmitted via a droplet or breathing to the endpoint cell when infected while it was associated with diarrhea as a common sign of infections with the coronavirus. Up to 30% of MERS-CoV patients and 10.6% of SARS-CoV patients have it identified (D'amico et al., 2020). There were some studies that reported interesting results on the estimated number of ingested SARS-CoV-2 genomic copies/dip (one swim = 32 mL) is 4.6×10^{-7} to 80.5. It was determined that the yearly probability of infection was greater than 1/10,000 ($> 9 \times 10^{-12}$ to 5.8×10^{-1}). Furthermore, the study suggested using QMRA to assess and rank potential health hazards associated with swimming in tainted waterways during the COVID-19 pandemic (Tyagi et al., 2021).

2.6 Quantitative Microbial Risk Assessment (QMRA)

QMRA is a tool that estimate the risks to human health by using dose-response models for particular pathogens and assessment for various exposure scenarios (Haas et al., 2014). QMRA can facilitate quantitative risk for drinking water suppliers worldwide (Schijven et al., 2011) as well as predicts human health effects from stormwater pathogens (McBride et al., 2013). Additionally, QMRA is also essential for monitoring and examining water quality and can predict the water quality trend for decision of water use for domestic, irrigation, and recreation.

World Health Organization has developed QMRA application for water safety management. The QMRA framework consists of four steps: 1) articulation of the problem; 2) assessment of exposure; 3) evaluation of the health impacts; and 4) risk characterization (Organization, 2016). The National Academy's four-tiered approach—hazard identification, dose-response, exposure assessment, risk characterization, and risk management—can serve as a model for these frameworks

(Haas et al., 2014). In this study, applied some details of 4 steps to develop the study and focus on achieving the objective and expected outcome. However, some studies found that an activity including swimming, canoeing, motorboating, and fishing are rather prevalent in surface waters. In addition, compared to non-water recreators, water recreators have a higher incidence of acute gastrointestinal disorders along with other ailments like respiratory, ear, eye, and skin complaints (DeFlorio-Barker et al., 2018). Several studies showed that a risk assessment and many assumptions can be used to calculate acceptable risk. QMRA through swimming, boat trip, and fishing in recreation can be modified assumption from a previous study as the details below.

For NoV, the fractional Poisson model was used to compute the probability of infection (Pinf), which was 60% in the event of sickness given infection (Pill). (Vergara et al., 2016)

$$P_{\mathrm{inf}} = P(1 - e^{-\frac{\mathrm{dose}}{\mu}}) \text{ where } P = 0.722 \text{ and, } \mu = 1106 \qquad \ldots (4)$$

For SARS-CoV-2, the risk of infection use Tyagi et al. (2021) to comprehend potential SARS-CoV-2 exposure concerns from the aquatic environment, they concentrated on unintentional water consumption during leisure activities (swimming) as an exposure scenario (ingestion = 32 mL per dip/swim event). Furthermore, the result of (Tyagi et al.) according to the study, the amount of ingested SARS-CoV-2 varied from 4.6×10^{-7} to 80.5 genomic copies/dip (one swim being equivalent to 32 mL). The annual risk of infection was determined to be > 1/10,000 ($> 9 \times 10^{-12}$ to 5.8×10^{-1}). They use an assumption to calculated a dose-response model as below

$$P(d) = 1 - \exp^{-k*f*d} \qquad \ldots (5)$$

where P is the probability of infection following a single exposure at the dose, d is the ingestion dose per event, k is the likelihood that an infection will be caused by a single particle, and f depends on the scenario. It was assumed that d = 1 (Tyagi et al., 2021).

The study research defined scenarios from water activities by water quality index from ingestion during swimming recreation, consumption of lettuce irrigated with surface water containing noroviruses and SARS-CoV-2, and boating transportation along the Canal. Swimming recreation and boating transportation were used exposure assessment parameters and dose-response assessment from (Van Abel et al., 2017).

For swimming recreation and boating transportation following equation 6:

$$D = C \times \frac{1}{R} \times 10^{-\mathrm{Inact}} \times \mathrm{Inf} \times \mathrm{Vcons} \qquad \ldots (6)$$

Quantifying the mean dose (genomes per day or event) of D of equation 5 in water for each exposure involves accounting for the following factors: the amount of water consumed (Vcons), the recovery efficiency of the detection method (R), the percentage of infectious viruses (Inf), the amount of sunlight inactivation (Inact), and the concentration of SARS-CoV-2 and norovirus in the water (C).

For consumption of lettuce irrigation following equation 7:

$$d_s = 10^{(\log10(C_w x V_{surf}) - R_s - R_T - R_{wash})} \times I \qquad \ldots (7)$$

where RT is the quantity of virus reduction achieved during the interval among harvest and consumption, Rwash is the amount of surface viruses due to washing with water, I is the amount of lettuce consumed, and Cw is the mean concentration of NoV in surface water, Vsurf is water that clings to the surface through sprinkler irrigation, and Rs is the amount of virus reduction on the surface due to exposure to UV and high temperatures in the field (Sales-Ortells et al., 2015).

In this QMRA, to account for uncertainty in the predicted outcomes, NoV dose response models were used Beta – Poisson model following equation 8.

$$\text{Beta} - \text{Poisson model: } p(d) = 1 - [1 + d/N_{50}\ (2^{\frac{1}{\alpha}-1}] - \alpha \qquad \ldots (8)$$

For SARS-CoV-2 was used exponential model with the following equation:

$$\text{Exponential model: } p(d) = 1 - \exp\left(-\frac{d}{k}\right) \qquad \ldots (9)$$

In terms of dose-response model selection to assess NoV and SARS-CoV-2 risk pose in surface water considered on data distribution and reference dose. Several research frequently applies the Exponential model and Beta-Poisson (Pujol et al., 2009) highlighted the differences between the Exponential model and Beta-Poisson on a dataset for rotavirus. They found that the cumulative dose model and the exponential dosage model both tended to overestimate the likelihood of infection. The Exponential model grows too quickly in comparison to a one-infection probability, making it impossible for the model to sustain a non-zero or non-one infection probability. In contrast, the Beta-Poisson model is not constrained by these factors. A wider variance range is provided by its slower convergence to 1. Because adults between the ages of 18 and 45 are likely to have been exposed to different rotaviruses on multiple occasions, the Beta-Poisson model fit the data more closely statistically than the Exponential model. This heterogeneity in susceptibility flattens dose-response curves beyond what exponential dose-response models can account for.

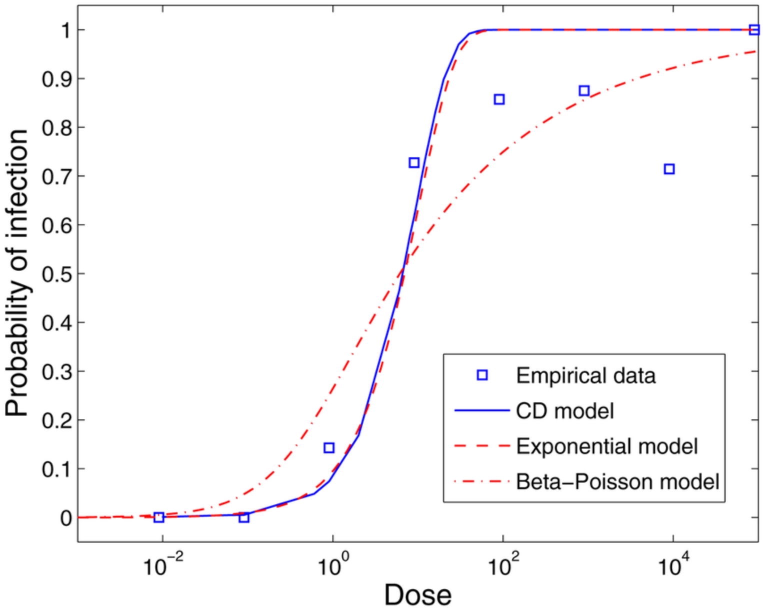


Figure 7 Dose-response curves based on the Exponential Model (EM), the Beta-Poisson model (BP) and the Cumulative Dose model (CD) compared to the experimental dataset for Rotavirus (squares) (Pujol et al., 2009)

Therefore, a dose-response based on the exponential model and the Beta-Poisson model, as previously described, should be used to select a best-fit model for the research data. This selection aligns with the QMRA methodology, including specific parameters for quantitatively assessing microbial risk.

Chapter
3 Methodology

This study was conducted the methodology 6 steps follows;

Step 1 Study sites and water sample collection by surveyed a location and defined a sampling site using based on the historical surface water quality data from the Pollution Control Department (PCD), the Maha Sawat Canal and Tha Chin River were a representative study site. Regarding to water sample collection was defined 9 sampling site and 9 months from December 2021 to August 2022 cover dry and wet season, used the standard method of Announcement of the National Environment Board No. 8 (1994) regarding setting water quality standards in surface water sources, Thailand.

Step 2 Virus concentration and genome extraction methods by using an Amicon® Ultra-15 centrifugal filter device for virus concentration extraction and using a QIAamp Viral RNA Mini Kit for for viral RNA extraction.

Step 3 Quantification virus by RT-qPCR assay for norovirus GI, GII, and SARS-CoV-2 were carried out utilizing the primers, probes, and PCR amplification conditions on the Quant StudioTM 6 Flex Real-Time PCR System. Quant Studio Design & Analysis software was used to analyze the RT-qPCR data.

Step 4 The water quality used the water quality data in the same sampling site from Thai Climate Change and Environmental Research Center, Mahidol University (MU), and the PCD.

Step 5 The water quality index calculation utilized the water quality index (WQI) assessment provided by Thai Pollution Control Department (PCD) version 2010.

Step 6 Quantitative Microbial Risk Assessment utilizes the US EPA and World Health Organization (WHO) as benchmarks for the four-tiered methodology recommended by the National Academy, which includes hazard identification, dose-response, exposure assessment, risk characterization, and risk management.

Therefore, the six steps of the framework were summarized briefly in Figure 8.

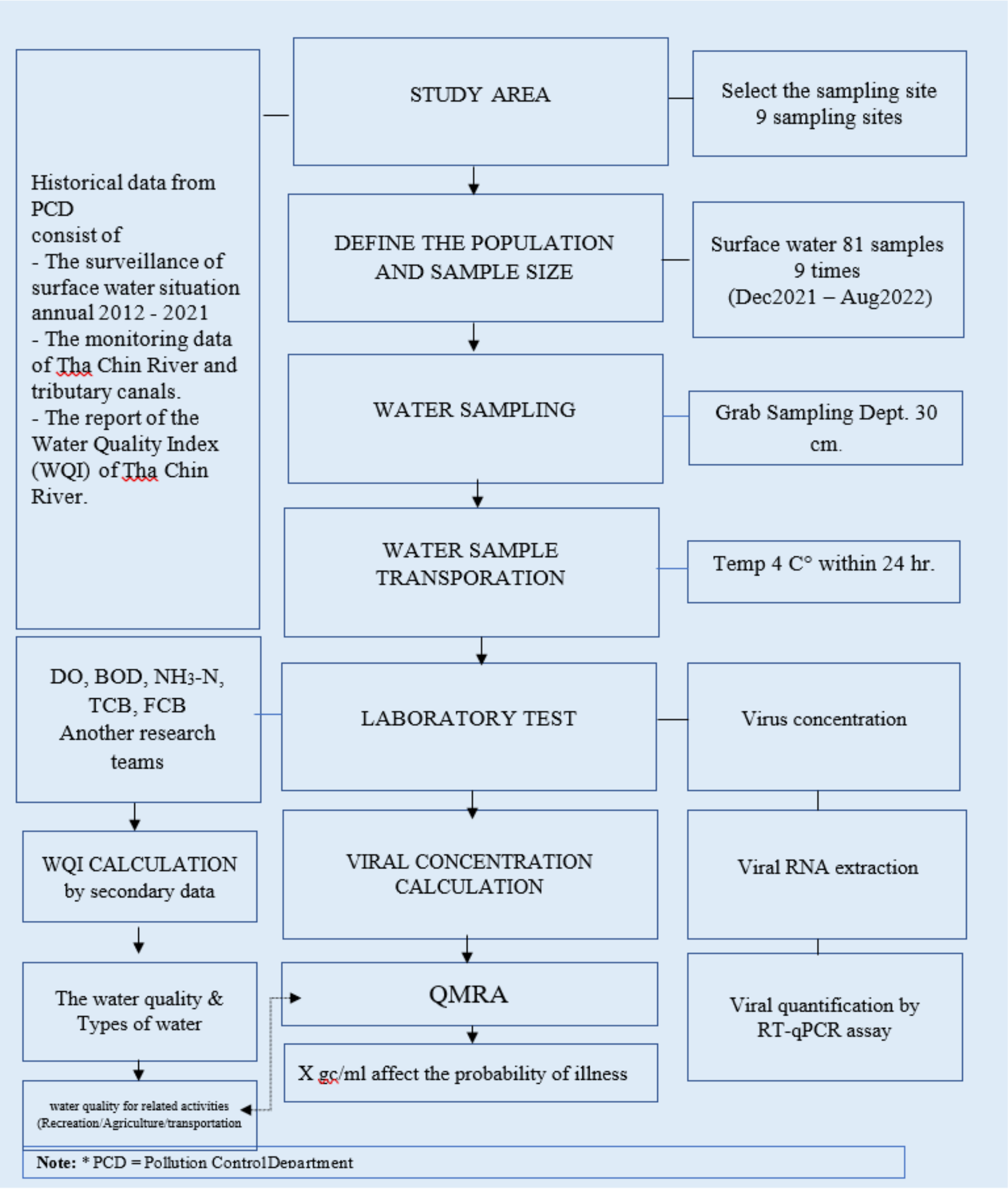


Figure 8 The methodology conceptual framework

3.1 Study sites and water sample collection

A total of 81 surface water samples were collected from 9 water sampling sites in the Maha Sawat Canal (MSC) of Nonthaburi, Bangkok, and Nakhon Pathom Provinces, Thailand, during 9 sampling events in the dry season (December 2021 to April 2022) and the wet season (May to August 2022) (Figure 9). The MSC was selected to the representative area studied from tributaries of That Chin River by 28 kilometers distance. The location of the sampling site and possible main source of wastewater as (Table 5). The samples from Maha Sawat canal were collected by grab-sampling method in surface water at depths of 0.3 meters, collected water samples before noon. Within eight hours, the two-liter samples were collected in sterile plastic containers and shipped to the appropriate laboratories on ice. Before undergoing additional examination, the samples were kept at 4°C for no more than 24 hours.

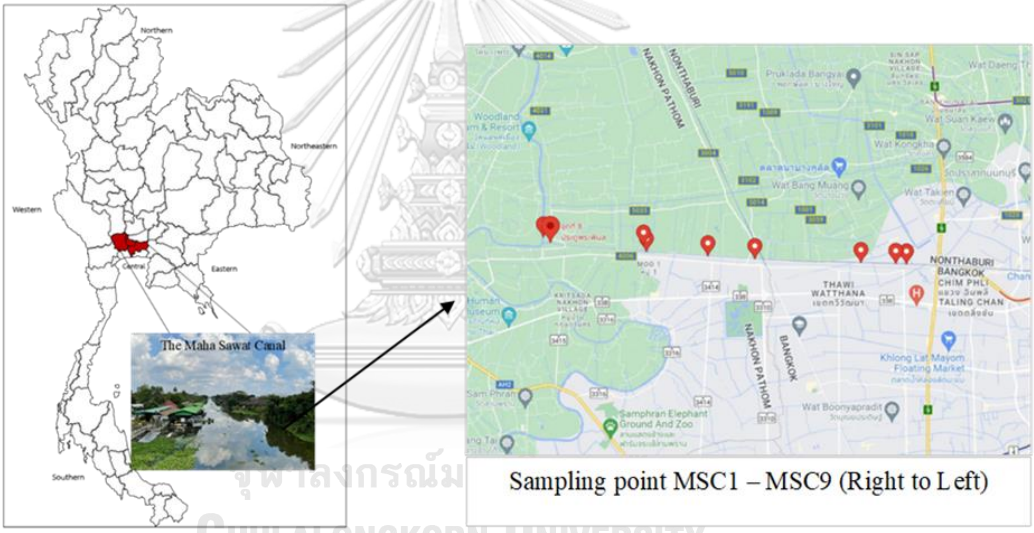


Figure 9 Sampling locations of Maha Sawat Canal in Nonthaburi (MSC1–2, Bangkok (MSC3-4)) and Nakhon Pathom (MSC5-9)

Table 5 Sampling site location in Maha Sawat Canal

Sampling site	Location	Possible main source of wastewater	X	Y
1	Water gate 1 (Initiation Site)	Domestic wastewater	650863.626	1526260.939
2	Water gate 2	Domestic wastewater	650313.919	1526282.334
3	Temple 1	Domestic wastewater	648535.820	1526321.264
4	Temple 2	Domestic wastewater	643120.307	1526481.353
5	Hospital	Hospital wastewater	640708.091	1526621.803
6	Floating market	Commercial, restaurant, market wastewater	637556.852	1526826.147
7	Homestay	Domestic wastewater, Farming	637428.033	1527145.009
8	Watergate3	Domestic wastewater	632660.149	1527200.104
9	than Chin River (End Site)	Domestic, commercial, restaurant, market wastewater	632285.9305	1527530.123

3.2 Virus concentration and genome extraction methods

Within eight hours after the sample collection, 45 to 100 mL of the samples were processed using the viral concentration method. Using an Amicon® Ultra-15 centrifugal filter system (Merck Millipore Ltd., Burlington, MA, USA) with a 30 kDa molecular weight cut-off, 20 ml of sample were centrifuged at 4200 ×g for 10 min at 4°C. Three to five centrifugations of the sample were performed until the membrane began to clog. The QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) was utilized to extract viral RNA from the 140 µL concentrated samples. Until further examination, the 60 µL isolated genome was kept between -30 and -20 °C.

3.3 Quantification virus by RT-qPCR assay

RT-qPCR assays for norovirus GI, GII, and SARS-CoV-2 were carried out utilizing the primers, probes, and PCR amplification conditions on the Quant Studio^{TM} 6 Flex Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) listed in Table 6. For SARS-CoV-2, 2.5 µL of TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific), 0.3 µL of each of the 10 µM forward and reverse primers, 0.2 µL of each of the 10 µM hydrolysis probe, 2.5 µL of the extracted template, and 3.4 µL of sterile water made up the 10 µL

RT-qPCR reaction mixture for the N1 and N2 multiplex assay. For norovirus, the 10 µL RT-qPCR reaction mixture contained 2.5 µL of TaqMan Fast Virus 1-Step Master Mix, 0.5 µL of forward and reverse primers at 10 µM each, 0.25 µL of hydrolysis probe at 10 µM, 2.5 µL of extracted template, and 3.75 µL of sterile water. Using Quant Studio Design & Analysis software (Applied Biosystems), the RT-qPCR findings were examined. The threshold values were manually adjusted to 0.04 for noroviruses and 0.115 for N1 (SARS-CoV-2) viruses, while the baseline was adjusted automatically. The Qubit 3.0 fluorometer (Thermo Fisher Scientific) was used to quantify the standard curves, which were created using linearized synthetic plasmid standards (GeneArt®, Invitrogen, Thermo Fisher Scientific). Viral quantification was performed on SARS-CoV-2, norovirus GI, and GII positive water samples that were of sufficient volume and had cycle threshold (Ct, the number of cycles needed for the fluorescent signal to cross the background threshold level) values of LOD 38.5, LOQ 37.5 for SARS-CoV-2, and LOD 45, LOQ 40 for norovirus. The gene copies computation for the data below the limit of quantification (LOQ) based on the N1 and norovirus standard curves.

Table 6 Sequences of primers and probes and qPCR conditions for noroviruses and SARS-CoV-2.

Assay	Primer /Probe	Sequence (5´-3´)	Amplification condition	Reference
Norovirus GI	**Forward primer**	CGYTGGATGCGNTTYCATGA	5 min at 50 °C, and 20 s at 95 °C followed by 45 cycles of 3 s at 94 °C and 30 s at 56 °C	Modified from (Kageyama et al., 2003)
	Reverse primer	CTTAGACGCCATCATCATTYAC		
	TaqMan probe	VIC-AGATYGCGATCYCCTGTCCA-QSY		
Norovirus GII	**Forward primer**	CARGARBCNATGTTYAGRTGGATGAG	5 min at 50 °C, and 20 s at 95 °C followed by 45 cycles of 3 s at 94 °C and 30 s at 56°C	
	Reverse primer	TCGACGCCATCTTCATTCACA		
	TaqMan probe	6FAM-TGGGAGGGCGATCGCAATCT-QSY		
N1 gene of SARS–CoV–2	**Forward primer**	GAC CCC AAA ATC AGC GAA AT	15 min at 50 °C, and 20 s at 95 °C followed by 45 cycles of 3 s at 95 °C and 30 s at 55 °C	(Sangsanont et al., 2023)
	Reverse primer	TCT GGT TAC TGC CAG TTG AAT CTG		
	TaqMan probe	FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1		
N2 gene of SARS–CoV–2	**Forward primer**	TTA CAA ACA TTG GCC GCA AA	15 min at 50 °C, and 20 s at 95 °C followed by 45 cycles of 3 s at 95 °C and 30 s at 55 °C	
	Reverse primer	GCG CGA CAT TCC GAA GAA		
	TaqMan probe	HEX-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1		

3.4 The using the water quality by the secondary data

The study was used the water quality data in the same sampling site from Thai Climate Change and Environmental Research Center and Mahidol University (MU). Only NH_3-N using the surface water monitoring data from the PCD for the reliability. Regarding to the water quality methodology followed by Announcement of the National Environment Board No. 8 (1994) regarding setting water quality standards in surface water sources, Thailand as illustrated in Table 7.

Table 7 The five parameter and the method for laboratory test.

No.	Parameter	Method
1	DO	Azide Modification
2	BOD	Azide Modification at 20°C 5 Day
3	NH_3-N	Distillation Nesslerization
4	Total Coliform Bacteria	Multiple Tube Fermentation Technique
5	Feacal Coliform Bacteria	Multiple Tube Fermentation Technique

3.5 The water quality index calculation

This study utilized the WQI assessment provided by the PCD that used water quality data in the same sampling site from Thai Climate Change and Environmental Research Center and Mahidol University (MU). Moreover, the study was obtained NH_3-N because of inadequate NH_3-N field data for WQI calculation, PCD's surface water monitoring data at Maha Sawat Canal (MSC) station and Tha Chin River (TC13) station between 2 seasons, the dry season (December,2021 to April 2022) and the wet season (May to August, 2022). On the other hand, while researchers collected water samples, they investigated the minimum value of NH_3-N and therefore reported the same value in all of the studies, we applied PCD data for the reliability of NH_3-N.

Regarding to water quality parameters for WQI calculation namely DO, BOD, NH_3-N, TCB, and FCB by PCD-WQI calculation program were described by (Choo-In et al., 2015). According to Thai PCD's WQI that are included in the literature review, the score of PCD-WQI values were categorized into five rating categories: Excellent, Good, Fair, Poor, and Very Poor. Additionally, based on the kind of surface water quality standard, the Maha Sawat Canal's water quality analysis results were utilized to categorize the appropriate surface water resources for consumption as (Prakirake et al., 2009) demonstrated.

3.6 Quantitative Microbial Risk Assessment (QMRA)

The risk assessment of NoV GI and SARS-CoV-2 associated with risk of ingestion during boat transportation, swimming recreation, and lettuce consumption were done following QMRA protocol consisting of following steps.

Exposure assessment

The exposure pathways considered were for related with WQI of MSC, the exposure pathways of: (a) incidental exposure by ingestion during swimming, (b) exposure by ingestion during boat transportation, and (c) consumption of vegetables (lettuce) plant from irrigation water.

Scenario (a) and (b) ; In each exposure, the mean dose (genome copies (gc) per day or event) of norovirus and SARS-CoV-2 in water (D) is quantified based on the following parameters and equations: virus concentration in water (C), recovery efficiency of the detection method (R), proportion of infectious viruses (Inf), sunlight inactivation (Inact), and volume of water consumed (Vcons) (Van Abel et al., 2017). Table 8 provides a thorough breakdown of all exposure assessment parameters, which were computed using equation 6.

Scenario (c); Equation 7 illustrates the daily dose of virus on lettuce surface (dS) consumed by customers of the market when surface water irrigated lettuce is sold. where Vsurf is the water that adheres to the surface of lettuce during spray irrigation, and Cw is the concentration of NoV in surface water. Rwash is the reduction of surface viruses brought on by washing with water, I is the ingestion of lettuce, RT is the reduction of viruses attained during the interval between harvest and consumption, and Rs is the reduction of viruses on the surface brought on by exposure to UV and high temperatures in the field (Sales-Ortells et al., 2015). Table 9 provides a full list of all exposure assessment parameters.

Dose-response assessment

The risk of a response of infection and illness is estimated using the laboratory results of SARS-CoV-2 and norovirus from the water sample. Oracle Crystal Ball was used to generate statistics shape parameters and a goodness-of-fit analysis was used to determine each viral data set. Consumption of water represented as a lognormal distribution. The scenario involves ingestion while boating and swimming (Vergara et al., 2016). Equation 8 provides the fractional Poisson model that is used to compute the likelihood of illness given infection (Pill) and in which case the probability of infection (Pinf) for norovirus (Messner et al., 2014)

Based on the conditional likelihood that an individual would become ill if infected (Pill|inf), the probability of illness was determined using the formula Pill = Pill|inf \times Pinf. Based on the number of illnesses reported from the infected patients in a human challenge research, a triangular distribution (minimum = 0.3, maximum = 1, mode = 0.6) was used to estimate the likelihood of getting ill once infected (Pill|inf) (Teunis et al., 2008).

$$\text{Pill} = \text{PillIIinf} \times \text{Pinf}$$

In the QMRA of SARS-CoV-2, We had limited data on a dose-response model in the QMRA of SARS-CoV-2, which describes the association between the risk of infection and the viral exposure dosage used to measure the probability of catching a virus. This research will apply the construction of a dose-response model for SARS-CoV-2 while accounting for all of its uncertainty, given the fact that a SARS-CoV dose-response model utilizing intranasal infection has been developed (Kumar et al., 2021; Watanabe et al., 2010) equation 9 from Watanabe's model proposed an exponential model.

Table 8 Exposure assessment parameters for scenario (a) and (b)

Variable	**Definition**	**Units**	**Distribution**	**Value**	**Description**	**Reference**
C_{NoVGI}	Norovirus GI Concentration	gc/L	Lognormal	μ = 0.03 σ = 0.04	Mean = 0.02 gc/L	Collected & analyzed data
C_{SARS}	SARS-COV-2 Concentration	gc/L	Lognormal	μ = 0.007 σ = 0.009	Mean = 0.0007 gc/L	Collected & analyzed data
R_{low}	%recovery	%	Uniform	Min = 4.18 Max = 6.15	Wastewater	(Sangsanont et al., 2023)
R_{high}	%recovery	%	Uniform	Min = 26.71 Max = 65.71	untreated wastewater	(Ahmed et al., 2020)
Inact	Inactivation by sunlight	Log reduction	Uniform	Min = 1, max =3	NTU = 40 = 80	Best estimate; based on (Flannery et al., 2013) and (Lee & Ko, 2013)
Inf	% infectious (viable)	%	n/a	100	Conservative assumption	Best estimate
$V_{cons, RW, b}$	Consumption RW, boating	mL/hr	Triangle	Min = 0.1, mode = 3.9, max = 11.8	Mean = 1.9 ml/hr; based on canoeing	(Dorevitch et al., 2011) and (Sales-Ortells & Medema, 2014)
$V_{cons, RW, s}$	Consumption RW, swimming	mL/hr	Lognormal	μ = 2.92 σ = 1.43	Mean = 55 ml/Event	(Dorevitch et al., 2011) and (Sales-Ortells & Medema, 2014)
$t_{RW, b}$	Time spent boating	Hr/day	Triangle	Min = 1, mode = 2, max = 4	Mean = 2.1 hr/day	(Sales-Ortells & Medema, 2014)
$n_{RW, b}$	Numbe of recreation(boating) days per year	Days/yr	Uniform	Min = 1, max = 108	Boating can occur from 1 to 108 days per year	(Sales-Ortells & Medema, 2014)
$n_{RW, s}$	Numbe of recreation(swimming) events per year	Events/yr	Negative binomal	r = 1, λ = 0.04	Boating can occur from 1 to 108 days per year	(Sales-Ortells & Medema, 2014)

Table 9 Exposure assessment parameters for scenario (c)

Variable	Definition	Units	Distribution	Value	Description	Reference
Ct_{eff}	the concentration of NoV in tertiary effluent	gc/L	Gamma		Use only parameter	Collected & analyzed data
R_w	Log reduction due to tertiary treatment (alternative scenario)	Log10 Unit	PERT	0.57, 0.94, 1.30	Use min value	(Sales-Ortells et al., 2015)
R_s	in - field reduction of surface viruses	Log10 Unit	Uniform	Min = 1 Max = 2	the reduction of virus on the surface due to exposure to UV and high temperatures in the field	
R_T	reduction of viruses during transport and storage	Log10 Unit	Uniform	Min = 0 Max = 1		
R_{wash}	reduction of surface viruses due to washing	Log10 Unit	PERT	Min=0.1 Max=1.2	truncated	
I	the lettuce ingestion	g pppd	Lognormal	μ = 20.72 σ = 26.35 inf=0, sup=120	Mean = 1.0	
V_{surf}	water that clings to lettuce surface through sprinkler irrigation	mL/g	Lognormal	Min=-4.57 Max =0.50,	Mean = 0.006	

Risk characterization

The risk characterization of norovirus and SARS-CoV-2 were calculated by integration of the data on exposure, dose-response, and hazard identification to calculate the likelihood that the public health issue exists, its size, and its variability and uncertainty (Haas et al., 2014) for the QMRA programmed in the Oracle Crystal Balls application, 10,000 iterations of the probabilistic Monte Carlo analysis approach were used. Acceptable risk benchmarks are determined based on references from the World Health Organization and the US.EPA, which the US EPA (2012) defined the illness standard as 36 GI illnesses/1000 exposures, or 0.036 for NoV. The benchmark is considered low when it is less than <0.036. that (Ahmed et al., 2018) referenced in research also US.EPA benchmark for recreational water of NoV GI (0.036) (Fewtrell & Bartram, 2001), For SARS-CoV-2 defined a benchmark follows by (Dada & Gyawali, 2021) that compared risk using the WHO guideline's threshold of 1 illness per 1000 exposed individuals (0.001).

3.7 Statistical analysis

3.7.1 Descriptive data

The descriptive statistics were performed on the qualitative data and the results were presented by mean, standard deviation (SD), min-max, and median. Using this statistical analysis to present the data of the concentration of water quality five parameters (DO, BOD, NH_3-N, TCB, FCB), the WQI score (Overall, the dry and the wet season), the concentration of NoV and SARS-CoV-2.

3.7.2 Two – way ANOVA test

Two – way ANOVA test was used to analyzed the mean comparison between each concentration virus and water quality 5 parameters.

3.7.3 Monte Carlo analysis

Monte Carlo analysis was used a probabilistic approach (10,000 iterations) for the QMRA that programmed in the Oracle Crystal Balls application.

3.7.4 Spearman’s correlation coefficient

Spearman’s correlation was used to calculate the degree of correlation between the WQI variables, and the probability of illness. The degree of correlation between two variables were considered and interpreted as Table 10.

Table 10 The spearman’s correlation coefficients interpretation according to (Chan, 2003)

Degree of correlation	Interpretation
1 or -1	a perfect relationship
0.8 to 0.9 (-0.8 to -0.9)	a very strong relationship
0.6 to 0.7 (-0.6 to -0.7)	a moderate relationship
0.3 to 0.5 (-0.3 to -0.5	a fair relationship
0.1 to 0.2 (-0.1 to -0.2)	a poor relationship
0	no relationship

Chapter 4
Result and discussion

4.1 The prevalence of noroviruses and SAR-CoV-2

4.1.1 The detection rate of NoV GI and SARS-CoV-2

This study revealed that the detection rate of NoV GI and SARS-CoV-2 in surface water from Maha Sawat canal (MSC). The detection rate was 41.9% (34/81) and 9.9% (8/81) respectively. The data was categorized into two seasons: the dry season (December 2021 to April 2022) and the wet season (May to August 2022). The results showed that the prevalence of viruses was found higher in the dry season. Additionally, NoV had a higher positive detection rate than SARS-CoV-2 during the overall study period and within each season. The study found lower concentrations of the NoV in water environment during the wet season might slightly a high NoV concentration in the dry season was close consistent with previous studies (Ahmed et al., 2013; Grøndahl-Rosado et al., 2014). This study, SARS-CoV-2 prevalence is higher in the dry season, although (Bivins et al., 2020) described SARS-CoV-2 persistence in surface water related to low temperatures but It cannot be concluded that SARS-CoV-2 always prevalent in surface water in dry season (Table 11).

Table 11 Detection of Norovirus GI (NoV GI) and SARS-CoV-2 in surface water samples from the Maha Sawat Canal, Thailand

			Number of detections			
	Total number of virus detection		**Dry season (December,2021 – April,2022)**		**Wet season (May – August,2022)**	
Sampling site	**NoV GI**	**SARS-CoV-2**	**NoV GI**	**SARS-CoV-2**	**NoV GI**	**SARS-CoV-2**
1 (n=9)	5/9	0/9	3/5	0/5	2/4	0/4
2(n=9)	3/9	1/9	2/5	1/5	1/4	0/4
3(n=9)	4/9	1/9	4/5	1/5	0/4	0/4
4(n=9)	6/9	2/9	3/5	1/5	3/4	1/4
5(n=9)	6/9	1/9	5/5	1/5	1/4	0/4
6(n=9)	4/9	1/9	2/5	1/5	2/4	0/4
7(n=9)	3/9	0/9	3/5	0/5	0/4	0/4
8(n=9)	2/9	1/9	1/5	1/5	1/4	0/4
9(n=9)	1/9	1/9	1/5	1/5	0/4	0/4
Total (81)	**(34/81)** **41.9%**	**(8/81)** **9.9%**	**(24/45)** **51.3%**	**(7/45)** **15.6%**	**(10/36)** **27.8%**	**(1/36)** **2.8%**

Note; Number of detections consist of the total number of each virus sample 81, the dry season each virus sample 45, and the wet season each virus sample 36.

4.1.2 The concentration of positive virus of NoV GI and SARS-CoV-2

In the MSC, the concentration of NoV ranged from approximately 7.12×10^3 to 68.90×10^3 gc/L of overall events. Specifically, in dry season, NoV GI concentrations were between 7.12×10^3 to 68.90 ×10^3 gc/L, and in the wet season, they ranged from 12.56×10^3 to 36.86 ×10^3 gc/L. For SARS-CoV-2, 8 positive samples showed concentrations ranging from 4.99×10^3 to 10.48×10^3 gc/L overall, with 4.99×10^3 to 10.48×10^3 in the dry season, and 5.04×10^3 gc/L in the wet season as illustrated in Figure 10. Therefore, NoV GI concentrations were higher in the dry season than in the wet season as prevalence study. Also, SARS-CoV-2 had high concentration in the dry season rather than the wet season may supported the research assumption that during the COVID-19 epidemic, surface water quality assessing by WQI did not represent the spread of SARS-CoV-2 in the surface water.

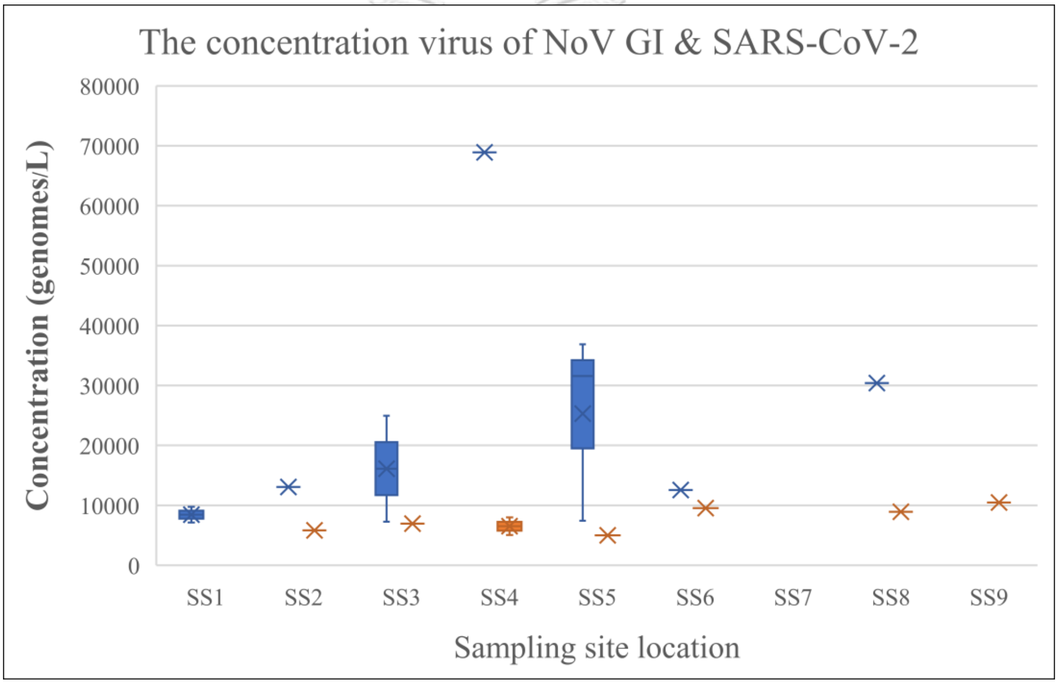


Figure 10 The concentration virus of NoV GI and SARS-CoV-2 in the Maha Sawat Canal and the Tha Chin River

Note: Source of wastewater from sampling site 1-9; SS1-SS4 (Domestics), SS5(Hospital), SS6(Commercial, Restaurant, Market), SS7(Domestic, Farming SS8 (Domestic, Office), SS9(Domestic, Office, Commercial, Restaurant, Market), concentration viruses were shown only positive virus that could be quantify by NoV GI (11/81), and SARS-CoV-2 (8/81).

The mean viral concentrations at each sampling site were depicted in Figure 10, sampling site 4 showed the highest concentration of norovirus GI with the concentration of 68.90 $\times 10^3$. This elevated concentration at sampling site 4 might be due to the contamination from the untreated domestic sewage but found positive detection rate 1.23% of total sample (1/81) while sampling site 5 most detection rate. SARS-CoV-2 concentration was relatively low across all sampling sites, which might be indicative of its lower prevalence in wastewater. Therefore, between sampling site 3 to 5 may had the source of possible pollution, the researcher lack of survey data about source of truly water pollution which may considered by the location found that the hospital, the market, and the community might poor wastewater management affected concentration was high than other sampling site.

4.2 The water quality

The water quality using the secondary data in the same sampling site from the Thai Climate Change and Environmental Research Center, Mahidol University (MU), and the PCD consist of Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Ammonia (NH_3-N), Total Coliform Bacteria (TCB), and Fecal Coliform Bacteria (FCB) compared with benchmark of surface water type 3 by Announcement of the National Environment Board No. 8 (1994) regarding setting water quality standards details in Table 12.

Table 12 The benchmark of surface water type 3

The water quality parameter	Surface water type 3 Benchmark
DO	≥ 4.0 mg/l
BOD	≤ 2.0 mg/l
NH_3-N	≤ 0.5 mg/l
TCB	≤ 20,000 MPN/100 ml
FCB	≤ 4,000 MPN/100 ml

The water quality result shows that DO exceeds the benchmark by 32.1% (26/81), BOD exceeds the benchmark by 56.7% (46/81), NH_3-N exceeds the benchmark by 29.6% (24/81), TCB exceeds the benchmark by 100% (81/81), and FCB exceeds the benchmark 2.5% (2/81). Hence, TCB and BOD pollute surface water might recommend water treatment before use. Regarding to each parameter was described and the discussed follows as;

4.2.1 Dissolved Oxygen (DO)

Another metric used to assess whether the water's nutrients could sustain aquatic life was DO. The DO content was 4.5 mg/L on average. It was possible to see that the average DO concentration was discovered to be greater than the ideal DO concentration of 4.0 mg/L. In addition, considered each sampling site found that DO during the water gate, home-stay, and Tha Chin River better than other location.

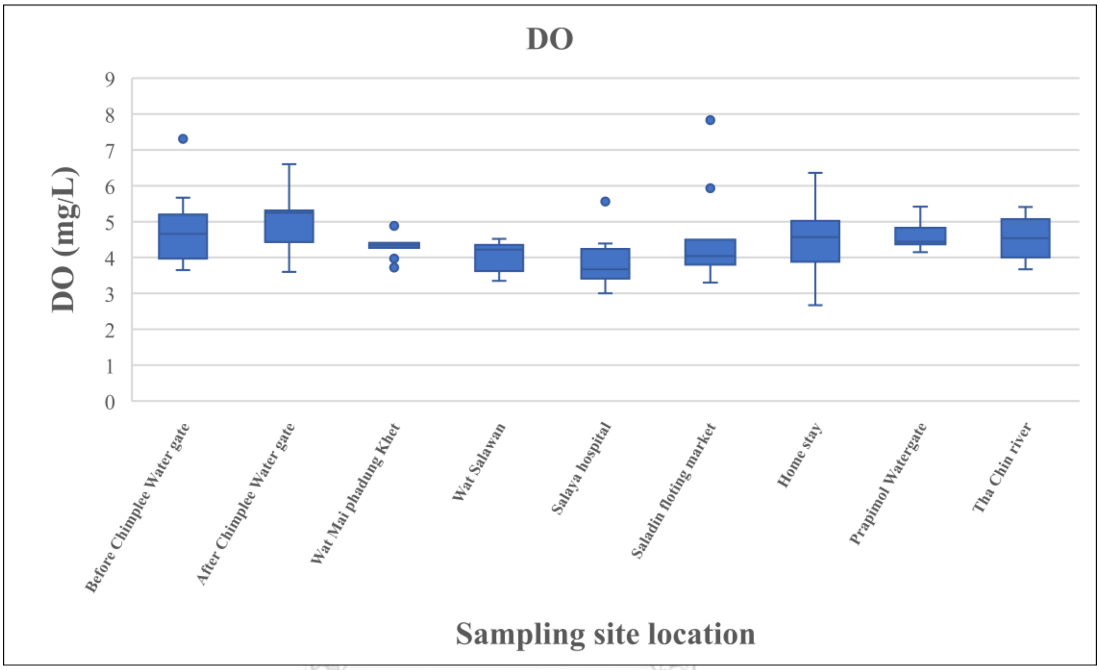


Figure 11 Dissolved Oxygen (DO)

Figure 11 shown that the 9 locations of 9 sampling sites, DO exceeds the benchmark in Wat phadung Khet, Wat Salawan, Salaya hospital, Saladin floating market.

4.2.2 Biochemical Oxygen Demand (BOD)

The amount of oxygen needed to stabilize household and industrial wastes has been measured using BOD. The average BOD content was determined to be higher than the surface water standard at 2.7 mg/L (BOD ≤2.0 mg/L), as evidenced by the overall BOD standard for the low-quality water. Therefore, it could be concluded that only BOD of the home-stay not over the standard.

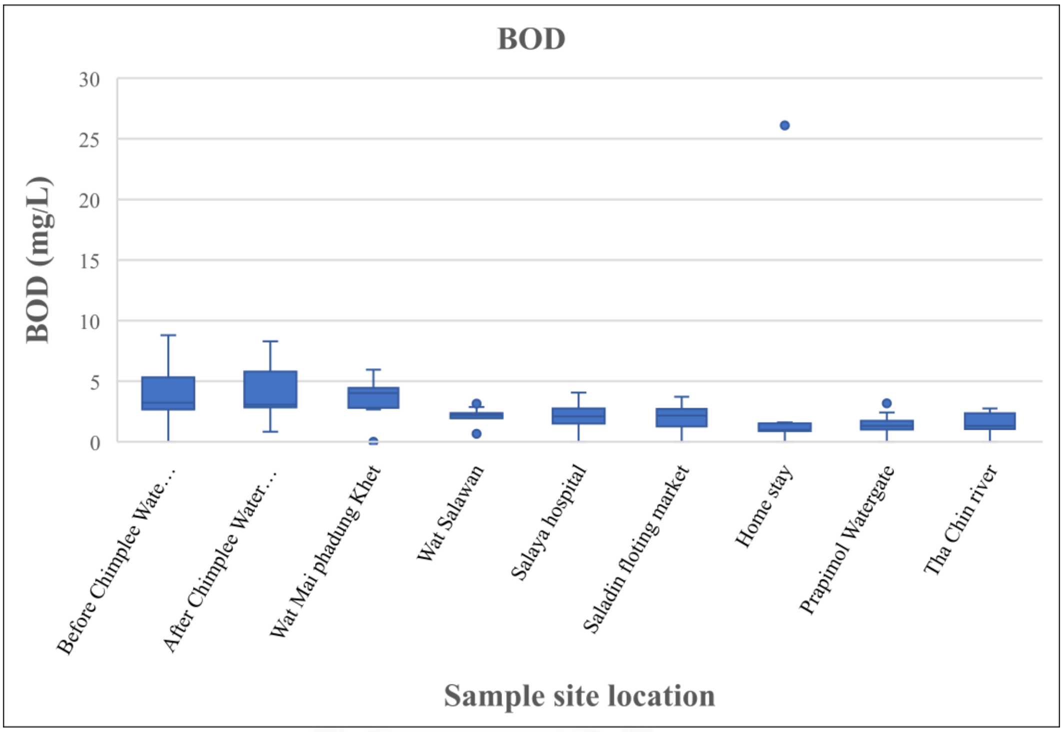


Figure 12 BOD in the MSC

Figure 12 shown that the 9 locations of 9 sampling sites, BOD exceeds the benchmark in before Chimplee water-gate, after Chimplee water-gate, Wat phadung Khet, Wat Salawan, Salaya hospital, Saladin floating market.

4.2.3 Ammonia (NH_3-N)

NH_3-N was used data from MSC station of PCD, Thailand. Limitation that could not use truly NH_3-N from study research. The average NH_3-N concentration was 0.3 mg/L. It could be good rang in the general water standard which significance to overview WQI score. In addition, Tha Chin River had NH_3-N no exceeds the benchmark.

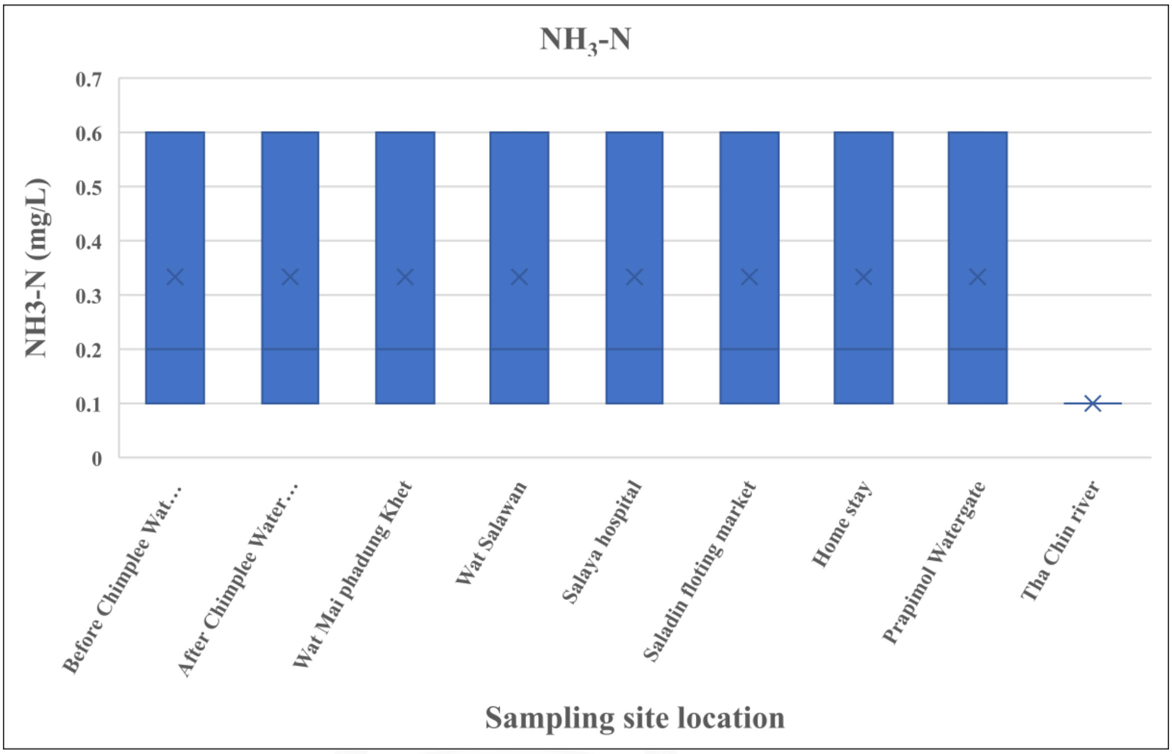


Figure 13 NH_3-N in the MSC

Figure 13 shown that the 9 locations of 9 sampling sites, BOD exceeds the benchmark in before Chimplee water-gate, after Chimplee water-gate, Wat phadung Khet, Wat Salawan, Salaya hospital, Saladin floating market, Home-stay, Prapimol water-gate.

4.2.4 Total Coliform Bacteria (TCB)

One significant factor affecting the Maha Sawat canal's quality is a high TCB concentration. The surface water criterion of ≤20,000 mg/L was exceeded by the average TCB concentration of 68,320 mg/L. This finding suggested that there is pollution in the water. These could be the outcomes of a number of things, including transportation, water hydraulics, and high wastewater domestic activity. In term of high TCB overall of the MSC, may should to treated water before consuming.

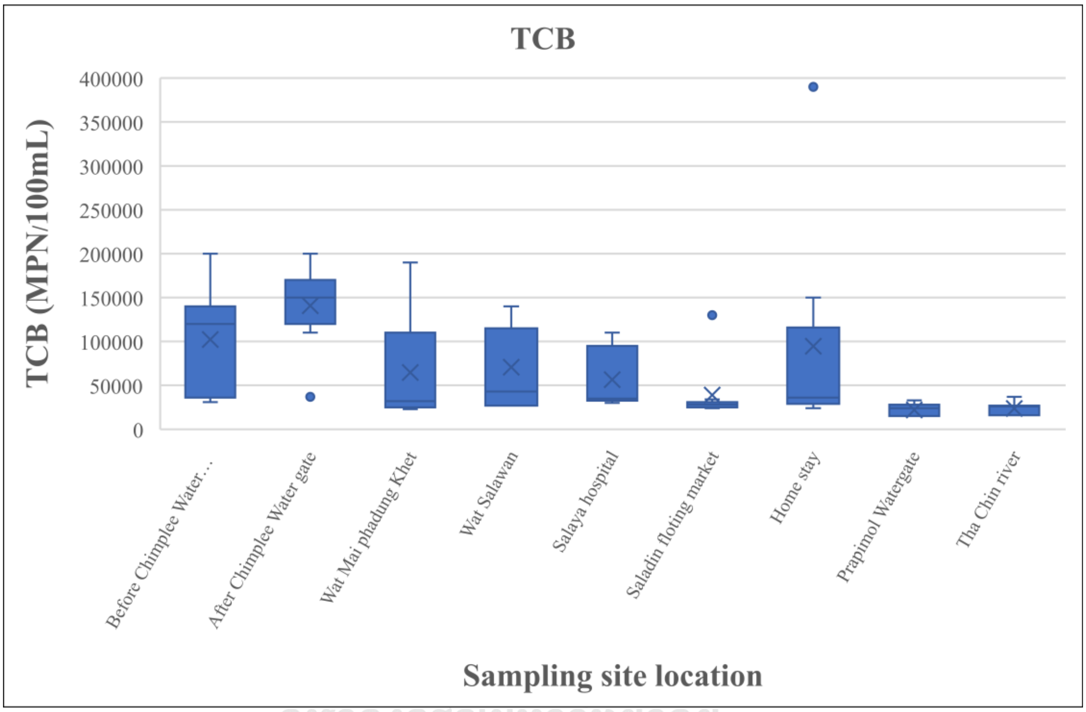


Figure 14 TCB in the MSC

Figure 14 shown that the 9 locations of 9 sampling sites, BOD exceeds the benchmark in before Chimplee water-gate, after Chimplee water-gate, Wat phadung Khet, Wat Salawan, Salaya hospital, Saladin floating market, Home-stay, Prapimol water-gate, and Tha Chin River.

4.2.5 Fecal Coliform Bacteria (FCB)

FCB was a crucial metric to show that elevated FCB concentrations can contribute to the etiology and pathophysiology of human diseases. There were 3,670 MPN/100 ml (less than 4,000 MPN/100 ml) of FCB on average, which overall concentration not exceeds the benchmark.

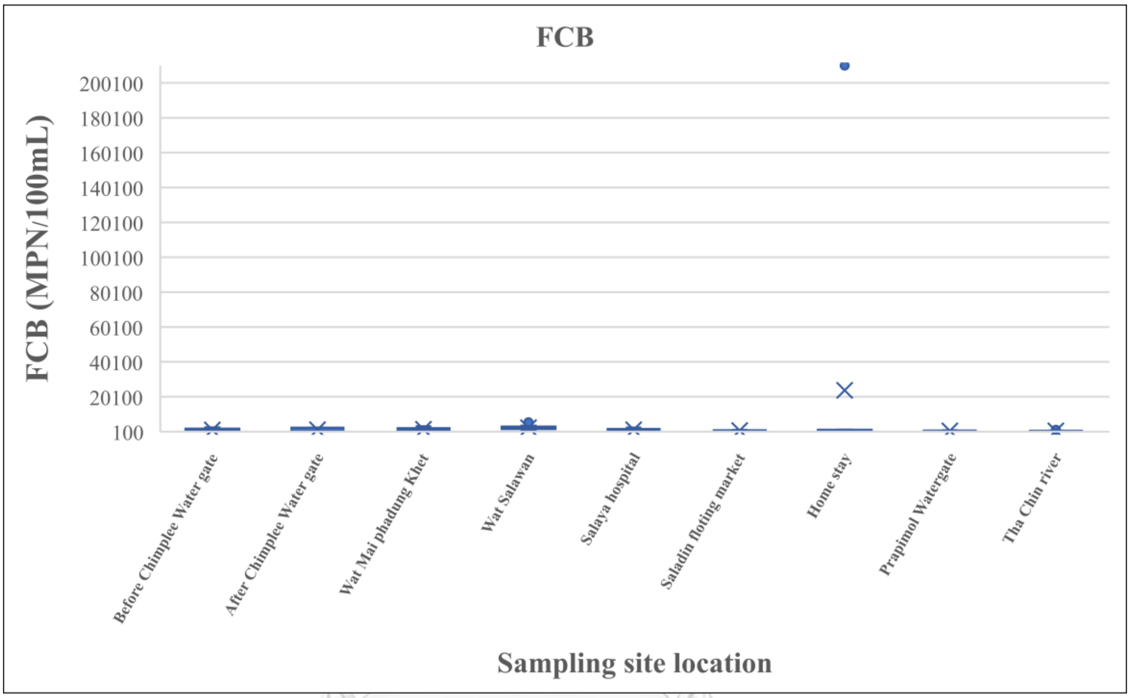


Figure 15 FCB in the MSC

Figure 15 shown that the 9 locations of 9 sampling sites, BOD not exceeds the benchmark in before Chimplee water-gate, after Chimplee water-gate, Wat phadung Khet, Wat Salawan, Salaya hospital, Saladin floating market, Home-stay, Prapimol water-gate, and Tha Chin River. Only the home-stay had high peak on 5,500 and highest peak 210,000 MPN/100 mL

The important parameters caused are water-polluted TCB and FCB which may not represent or relate to the prevalence of the virus in the canal. However, the water gate is important to flush in and flush out of water that may affect water quality. In addition, the water sampling point of Maha Sawat Canal (MSC) might be sufficient for normal situations while adding a sampling point to water quality monitoring during an emerging disease may benefit baseline data for the prediction of shedding emerging disease context.

4.3 The water quality index

The assessment of WQI in MSC from December 2021 to August 2022 showed a distribution ranking as follows: fair (42.0%), poor (33.3%), and good (24.7%) as shown in Table 13. The water quality on the Tha Chin River site seems to be better than the water quality toward the Chao Phraya River site. Notably, Tha Chin River demonstrated better water quality compared to other sampling sites based on Thai-PCD water quality data (http://iwis.pcd.go.th/index.php) in 2022, which rated it as good level (Score=87).

Table 13 The overall WQI of Maha Sawat Canal

Location	**Dry Season**					**Wet Season**			
	Dec-21	**Jan-22**	**Feb-22**	**Mar-22**	**Apr-22**	**May-22**	**Jun-22**	**Jul-22**	**Aug-22**
Before water-gate	Poor	Poor	Poor	Poor	Fair	Fair	Poor	Fair	Fair
After water-gate	Poor	Poor	Poor	Poor	Fair	Good	Poor	Poor	Fair
Temple	Poor	Poor	Poor	Poor	Poor	Good	Fair	Fair	Fair
Temple	Poor	Poor	Fair	Fair	Fair	Good	Fair	Fair	Poor
Hospital	Poor	Poor	Poor	Poor	Good	Good	Fair	Fair	Fair
Market	Poor	Poor	Fair	Poor	Good	Good	Fair	Fair	Fair
Homestay	Fair	Fair	Fair	Fair	Good	Good	Good	Good	Poor
water-gate	Fair	Fair	Fair	Fair	Good	Good	Good	Fair	Fair
River	Fair	Fair	Good	Good	Good	Good	Good	Good	Fair
Average	Poor	Poor	Fair	Fair	Fair	Good	Fair	Fair	Fair

Note: 1. Color box shown WQI level, red=poor, yellow=fair, green=good
2. Total number =81 which good number =20/81, Fair number= 34/81, Poor number= 27/81

The WQI was also used to assess the water quality based on the season and the results was depicted in Figure 16. Water quality in December 2021 to August 2022 the overall values were consistently ranked as fair level, both in the dry and wet seasons.

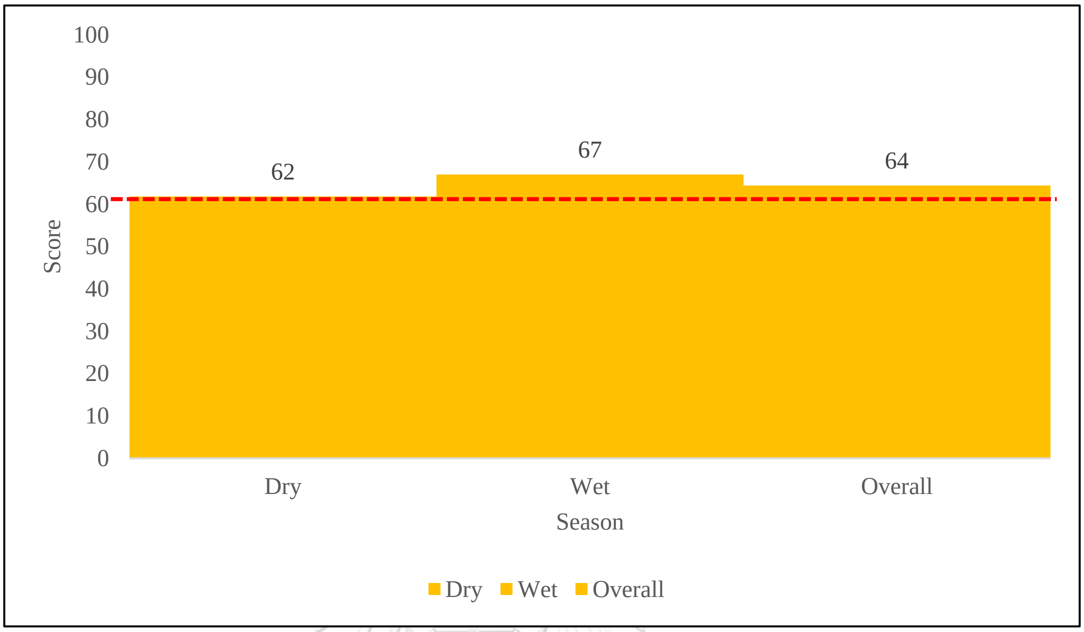


Figure 16 Seasonality of the water quality index on the MSC

Note; --- is MSC-WQI of Thai-PCD in 2022 (score=60, “Poor level”)

The water quality parameters, WQI, and suitability of water for each moth. The months of February, March, April, June, and July had fair water quality levels, while December, January and August had poor water quality level. Only May exhibited good water quality. Thus, the overall water quality over these nine months was considered suitable for agriculture and transportation. The lower scores in the WQI were primarily due to low levels of dissolved oxygen and high total coliform bacteria, among other factors. These parameters did not meet the water quality standards of Announcement of the National Environment Board No. 8 (1994) regarding setting water quality standards in surface water sources, Thailand, as detailed in Table 14.

Table 14 The mean of water quality index in Maha Sawat canal, Thailand.

Month	DO ≥ 6 (mg/L)	BOD ≤ 1.5 (mg/L)	NH_3-H ≤ 0.5 (mg/L)	TCB ≤ 5,000 (MPN/100ml)	FC ≤ 1,000 (MPN/100ml)	WQI		Suitable for
						Score	Value	
Dec,2021	5.7	3.5	0.5	102,555.6	1,100.0	58	Poor	Industry, transportation
Jan,2022	4.8	3.1	0.5	63,555.6	1,311.1	58	Poor	Industry, transportation
Feb,2022	4.7	2.9	0.5	82,888.9	577.8	63	Fair	Agriculture, transportation
Mar,2022	4.3	3.7	0.5	82,888.9	888.9	61	Fair	Agriculture, transportation
Apr,2022	4.4	1.7	0.1	57,444.4	866.7	69	Fair	Agriculture, transportation
May,2022	3.7	0.1	0.1	41,666.7	1,344.4	75	Good	Fisheries, and Recreation, transportation
Jun,2022	4.9	2.6	0.1	51,966.7	1,111.1	66	Fair	Agriculture, transportation
Jul,2022	4.2	1.9	0.2	41,555.6	1,300.0	66	Fair	Agriculture, transportation
Aug,2022	3.8	5.1	0.2	89,555.6	24,533.3	60	Poor	Agriculture, transportation
Average	4.5	2.7	0.3	68,230.9	3,670.4	64.1	Fair	Agriculture, transportation

Although the study had WQI level aligned Thai-PCD water quality data of the IWIS platform (http://iwis.pcd.go.th/index.php) in 2022, the WQI score in the study was higher score and had good water in the Chimplee Water-Gate (sampling site 8) and Tha Chin River (sampling site 9). Therefore, considered of the water sampling sites 1 connected from Cho Praya River to sampling site 9 Tha Chin River presented the water quality in the MSC might better when received water from Tha Chin River in the dry season (Figure 17).

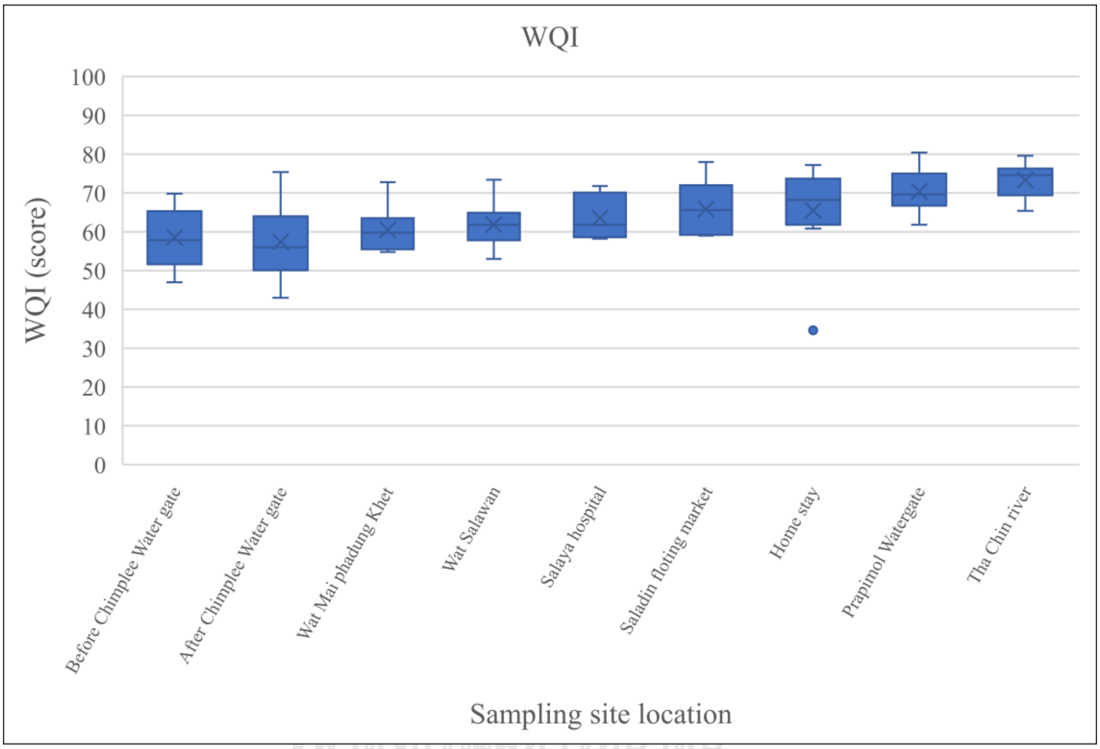


Figure 17 The WQI overall 9 locations of water sampling site in the MSC and Tha Chin River

Figure 17 shown that the 9 locations of 9 sampling sites, WQI relatively "fair" water quality status in before Chimplee water-gate, after Chimplee water-gate, Wat phadung Khet, Wat Salawan, Salaya hospital, Saladin floating market, Home-stay. WQI relatively "Good" water quality status in Prapimol water-gate, and Tha Chin River.

4.4 Quantitative Microbial Risk Assessment

4.4.1 Virus concentration

The study employed QMRA to estimate human health risks based on the concentrations of norovirus Genogroup I and SARS-CoV-2 found in positive water samples by NoV GI 11 of 81 samples, and SARS-CoV-2 8 of 81 samples, as detailed in Table 15. This assessment was applied across three distinct scenarios: swimming, boat transportation, and consumption of vegetables (lettuce) plant from irrigation water. The assessment utilized overall concentration, as well as separate analyses for dry, and wet seasons, to comprehensively evaluate the associated risks.

Table 15 The concentration of norovirus and SARS-CoV-2

Sample		**NoV GI**	**SARS-CoV-2**
Date detected	**Sampling site**	**Final concentration (gc/L)**	**Final concentration (gc/L)**
8/Dec/2021	SS1 (Water gate 1)	9.76 x10^{3}	-
	SS5 (Hospital)	31.58 x10^{3}	-
	SS8 (Water gate 3)	-	8.91x10^{3}
	SS9 (Tha Chin River)	-	10.48 x10^{3}
5/Jan/2022	SS1 (Water gate 1)	7.12 x10^{3}	-
	SS3 (Temple 1)	-	6.94 x10^{3}
9/Feb/2022	SS3 (Temple 1)	24.96 x10^{3}	-
	SS4 (Temple 2)	68.90 x10^{3}	-
8/Mar/2022	SS2 (Water gate 2)	-	5.83 x10^{3}
	SS5 (Hospital)	7.44 x10^{3}	4.99x10^{3}
5/Apr/2022	SS3 (Temple 1)	7.27 x10^{3}	-
	SS4 (Temple 2)	-	7.98 x10^{3}
	SS6 (Floating market)	-	9.54 x10^{3}
5/May/2022	-	-	-
8/Jun/2022	-	-	-
7/Jul/2022	SS4 (Temple 2)	-	5.04 x10^{3}
	SS5 (Hospital)	36.86 x10^{3}	-
3/Aug/2022	SS2 (Water gate 2)	13.07 x10^{3}	-
	SS6 (Floating market)	12.56 x10^{3}	-
	SS8 (Water gate 3)	30.40 x10^{3}	-
MIN		**7.12 x10^{3}**	**5.04 x10^{3}**
MAX		**68.90 x10^{3}**	**10.48 x10^{3}**
AVERAGE		**22.72 x10^{3}**	**7.46 x10^{3}**

Note: N of NOV positive = 11, N of SARS-CoV-2 positive = 8

4.4.2 Risk estimation from the QMRA

Scenario (a) incidental exposure by ingestion during swimming

The probabilities of infection were observed depending on the type of exposure, Norovirus GI, SARS-CoV-2, and dose-response model used the exposure pathways of incidental exposure by ingestion during swimming recreation. The probability of infection of NoV GI was overall 0.492 in the dry and wet season. The probability of illness of NoV GI was was overall 0.148 in the dry and wet season. The probability of infection of SARS-CoV-2 was 0.642 (overall), 0.653 (dry season), and 0.500 (wet season). The probability of illness of SARS-CoV-2 was 0.019 (overall), 0.020 (dry season), and 0.015 (wet season). (Table 16). When compared to the U.S.EPA benchmark for NoV GI (Fewtrell & Bartram, 2001), the risk of NoV exceeds benchmark. For SARS-CoV-2, a benchmark is defined according to (Dada & Gyawali, 2021) that compared the risk against a threshold of 1 illness per 1000 (0.001) exposed individuals, as specified in the WHO guideline. Based on this, SARS-CoV-2 presents a risk than the set benchmark. Moreover, both NoV GI and SARS-CoV-2 in the probability of infectious context relative to risk during one swimming event in the Maha Sawat Canal.

Table 16 The QMRA results for recreation via ingestion (Swimming) in the Maha Sawat Canal

Result	Units	Norovirus GI			SARS-CoV-2		
		DRY	WET	OVERALL	DRY	WET	OVERALL
Concentration in water	**gc/mL**	22.43	23.22	22.72	7.70	5.04	7.46
Dose	**Genomes /Event**	1.23×10^{6}	1.28×10^{6}	1.25×10^{6}	4.24×10^{5}	2.77×10^{5}	4.10×10^{5}
Probability of infectious	**event**	0.492	0.492	0.492	0.653	0.500	0.642
Probability of illness	**event**	**0.148**	**0.148**	**0.148**	**0.020**	**0.015**	**0.019**

Scenario (b) incidental exposure by ingestion during boat transportation

The probabilities of infection were observed depending on the type of exposure, Norovirus GI, SARS-CoV-2, and dose-response model used the exposure pathways of incidental exposure by ingestion during transportation. The probability of infection of NoV GI was overall 0.419 dry, and wet season. The probability of illness of NoV GI overall 0.126 in the dry and wet season. The probability of infection of SARS-CoV-2 was 0.035 (overall), 0.036 (dry season), and 0.024 (wet season). The probability of illness of SARS-CoV- overall 0.001 in the dry and wet season (Table

17). When compared to the U.S.EPA benchmark for NoV GI (Fewtrell & Bartram, 2001), the risk of NoV exceeds benchmark. For SARS-CoV-2, a benchmark is defined according to (Dada & Gyawali, 2021) that compared the risk to the WHO guideline's threshold of 1 sickness per 1000 exposed individuals. SARS-CoV-2 hence poses an acceptable risk relative to the benchmark.

Table 17 The QMRA results for transportation via ingestion (Boating) along the Maha Sawat Canal

Result	**Units**	**Norovirus GI**			**SARS-CoV-2**		
		DRY	**WET**	**OVERALL**	**DRY**	**WET**	**OVERALL**
Concentration in water	**gc/L**	22.43	23.22	22.72	7.70	5.04	7.46
Dose	**Genomes /Event**	4.26×10^4	4.41×10^4	4.32×10^4	1.46×10^4	9.58×10^3	1.42×10^4
Probability of infectious	**event**	0.419	0.419	0.419	0.036	0.024	0.035
Probability of illness	**event**	**0.126**	**0.126**	**0.126**	**0.001**	**0.001**	**0.001**

Scenario (c) consumption of vegetables (lettuce) plant from irrigation water

The type of exposure was observed to determine the probability of infection, norovirus GI, SARS-CoV-2, and dose-response model used the exposure pathways of consumption of vegetables (lettuce) plant from irrigation water. The probability of infection of NoV GI was 1.67×10^{-3} (overall), 1.65×10^{-3} (dry season), and 1.71×10^{-3} (wet season). The probability of illness of NoV GI was 2.25×10^{-1} (overall), 2.22×10^{-2} (dry season), and 2.29×10^{-1} (wet season). The probability of infection of SARS-CoV-2 was 1.93×10^{-9} (overall), 1.99×10^{-4} (dry season), and 1.31×10^{-4} (wet season). The probability of illness of SARS-CoV-2 was 2.94×10^{-7} (Overview), 3.03×10^{-7} (dry season), and 1.98×10^{-7} (wet season) (Table 18). When compared to the U.S.EPA benchmark for NoV GI (Fewtrell & Bartram, 2001), the risk of NoV exceeds benchmark. For SARS-CoV-2, a benchmark is defined according to (Dada & Gyawali, 2021) that compared the risk against a threshold of 1 illness per 1000 exposed individuals, as specified in the WHO guideline. Based on this, SARS-CoV-2 presents a lower risk than the set benchmark. Hence, NoV GI relative to the risk of consumed lettuce were planted with the MSC water transported to the market before washed with clean water and eaten.

Table 18 The QMRA results consumption of vegetables (lettuce) plant from irrigation water of the Maha Sawat Canal

Result	**Units**	**Norovirus GI**			**SARS-CoV-2**		
		DRY	**WET**	**OVERALL**	**DRY**	**WET**	**OVERALL**
Concentration in water	**gc/mL**	22.43	23.22	22.72	7.70	5.04	7.46
Dose	**g/day**	2.32×10^{-3}	2.41×10^{-3}	2.35×10-^{3}	7.98×10^{-4}	5.22×10^{-4}	7.73×10-^{4}
Probability of infectious	**event**	1.65×10^{-3}	1.71×10^{-3}	1.67×10^{-3}	1.99×10^{-9}	1.31×10^{-9}	1.93×10^{-9}
Probability of illness	**event**	**2.22×10^{-2}**	**2.29×10^{-1}**	**2.25×10^{-1}**	**3.03×10^{-7}**	**1.98×10^{-7}**	**2.94×10^{-7}**

The limitation of this study lies in the quantification of NoV GI and SARS-CoV-2 in water using RT-qPCR. This method cannot distinguish between infectious and non-infectious virus particles (Vergara et al., 2016). For assessment, the viral dose in the exposure pathway was considered 100% infectious, which might lead to an overestimation of the actual risk. Nonetheless, this approach is valuable for estimating the upper limit of risk associated with NoV GI and SARS-CoV-2 contamination.

In the present study, the quantitative PCR (RT-qPCR) norovirus and SARS-CoV-2 affiliated with the Maha Sawat Canal sampling sites were evaluated to determine the concentration at which this nucleic acid indicated significant health risk from exposure to surface water in the Maha Sawat Canal. The QMRA models were modified to assess an acceptable probability of illness (i.e., risk benchmark 0.036) of 36 GI illnesses per 1000 boating events (Ahmed et al., 2018) for the reference pathogens norovirus (NoV), SARS-CoV-2 modified a target probability of illness of 1 illness/1000 exposed individuals (i.e.,risk benchmark 0.001) for the reference pathogens SARS-CoV-2 (Dada & Gyawali, 2021).

The overall concentration of NoV GI in surface water was found to be 22.72×10^{3} gc/L. This level of contamination in MSC corresponded to a risk value greater than 0.036. Furthermore, the concentration of NoV GI during both the dry and wet season exceeded the established risk benchmarks, similarly to the overall NoV GI concentration. Consequently, there is a higher probability of illness in the consumption of vegetables (lettuce) plant from the irrigation water scenario from NoV GI in the wet season compared to the dry season. While incidental exposure by ingestion during swimming and boat transportation had a similar probability of illness in both of dry and wet seasons. This observation aligns with the findings of (Ahmed et al., 2013), who reported that the incidence of related illnesses associated with NoV peaks during the winter months.

In contrast, the overall concentration of SARS-CoV-2 in surface water from MSC was 7.46×10^{3} gc/L, which represented a low risk of the consumption of vegetables (lettuce) plant from the irrigation water scenario, a risk of 0.001 for the incidental exposure by ingestion during boat transportation scenario, and a risk exceeded the set of benchmarks of the incidental exposure by ingestion during swimming scenario. The previous study done by (Tyagi et al., 2021) using QMRA to assess SARS-CoV-2 exposure potential risks in the body of water revealed an infection risk higher than 1/10,000 annually risk.

This study used QMRA to described the risk of norovirus and SARS-CoV-2 in surface water, limited of research to demonstrated seasonality prevalence of SARS-CoV-2 in surface water while in 2021 the research of (Sangsanont et al., 2021) by collected water sample from January to April found that surface water may become contaminated by SARS-CoV-2 shedding in big to small centralized wastewater treatment plants. Despite (Mahlknecht et al., 2021) described that the presence of SARS-CoV-2 RNA in surface water 13% of the river samples were positive for viral RNA repeatedly during a SARS-CoV-2 peak phase between October 2020 and January 2021. Including to our study and several research detected SARS-CoV-2 relatively low might correlated with low risk of the consumption of vegetables (lettuce) plant from irrigation water and incidental exposure by ingestion during boat transportation, and a risk exceeded the benchmarks of incidental exposure by ingestion during swimming. In contrast with NoV GI was a higher risk of 3 scenarios, high concentration might relatively increase high risk associated with the previous study that mentioned it in the literature which was season, activities, and health risks.

4.5 The relationship between the water quality and concentration viruses

The relationship between the water quality and concentration analyzed the mean comparison between each concentration virus and water quality 5 parameters by Two – way ANOVA test, the result shown no significant of overall variables.

4.5.1 NoV GI and water quality parameters (DO, BOD, NH_3-N, TCB, FCB)

Testing the variance of virus concentration data with water quality results in Mahasawat Canal at the significance level = 0.05 by assuming that the average values of both virus concentrations and the 5 water quality parameters interaction within group and between group. The result shown no significant of NoV GI, SARS-CoV-2 and water quality all of 5 parameters both of within and between group therefore may considered the previous research (Gibson, 2014) about virus persistence in surface water than bacteria in term of bacteria could be not representative specific pathogens. The water quality may not adequately represent the concentration of virus in surface water, especially NoV GI no relationship with DO, BOD, NH_3-N, TCB, and FCB because *p*-value of two-way ANOVA test shown no significantly, details in table 19.

Table 19 Summary of NoV GI concentration and water quality (DO, BOD, NH_3-N, TCB, and FCB) in the MSC

Source	**df**	**Sum of squares**	**Mean Square**	**F**	***p*-value**
NoV GI & DO	1	1.196×10^7	11963364	0.113	0.738
NoV GI & BOD	1	2.831×10^5	283148	0.003	0.959
NoV GI & NH_3-N	1	1.204×10^8	120358089	1.135	0.290
NoV GI & TCB	1	2.687×10^8	268730440	2.534	0.116
NoV GI & FCB	1	1.281×10^8	128076613	1.208	0.275
Residuals	72	7.635×10^9	106041273		

4.5.2 SARS-CoV-2 and water quality parameters (DO, BOD, NH3-N, TCB, FCB)

Regarding to the relationship between SARS-CoV-2 and water quality parameters (DO, BOD, NH3-N, TCB, FCB) may not fully represent the SARS-CoV-2 positive concentration in surface water especially during the COVID-19. This study detected positive virus when compared the relationship between SARS-CoV-2 and DO, BOD, NH3-N, TCB, and FCB no significantly. Therefore, same sampling site, fair water quality status, and prevalence of SARS-CoV-2 detected in water sample no affected to these variables group as a details in table 20.

Table 20 Summary of SARS-CoV-2 concentration and water quality (DO, BOD, NH_3-N, TCB, and FCB) in the MSC

Source	**df**	**Sum of squares**	**Mean Square**	**F**	***p*-value**
SARS-CoV-2 & DO	1	2161020	2161020	0.371	0.544
SARS-CoV-2 & BOD	1	1235870	1235870	0.212	0.646
SARS-CoV-2 & NH3	1	22379	22379	0.004	0.951
SARS-CoV-2 & TCB	1	5916000	5916000	1.015	0.317
SARS-CoV-2 & FCB	1	351309	351309	0.060	0.807
Residuals	72	419469244	5825962		

4.6 The relationship between WQI and QMRA

4.6.1 The relationship between seasonality and mean concentration of NoV and SARS-CoV-2

The WQI seasonality indicated that the water quality is “fair level which of overall, dry and wet season. While the mean concentration virus of NoV GI was higher than SARS-CoV-2. Therefore, the relationship between WQI and Viruses shown suitable for agriculture, and transportation that could detected endemic and epidemic virus (Figure 18).

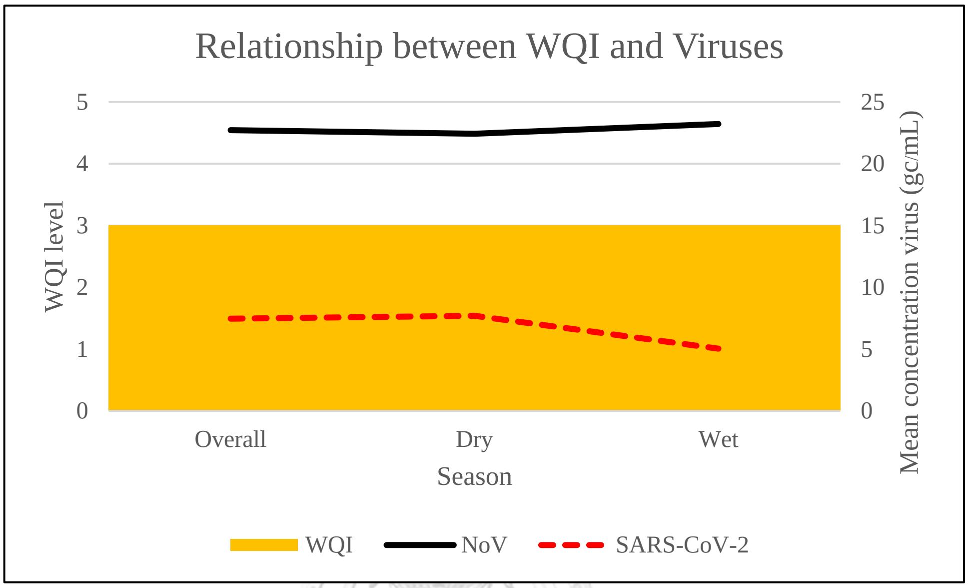


Figure 18 The relationship between WQI and virus concentration in seasonality

Figure 18 shown axe X = WQI level (1 to 5), axe Y = WQI, NoV GI, SARS-CoV-2, and axe Z = mean concentration virus (qc/mL). WQI overall, dry, and wet presented “fair” water quality status between NoV GI represented high mean concentration than SARS-CoV-2.

4.6.2 The relationship between seasonality and the probability of illness of NoV GI and SARS-CoV-2 on swimming, boating, and lettuce consumption

The WQI indicated that the water quality is suitable for recreation, agriculture, and transportation. However, the risk of NoV GI illness surpassed the benchmark (0.036) in the overall 9 months, the dry season, and the wet season-related water in each activity that mentioned it. While the risk of SARS-CoV-2 illness was low risk in the overall (9 months), dry, and wet seasons in boating, and consumed lettuce planted from water in the MSC, while the swimming recreation scenario in MSC exceeded the benchmarks (Figure 19- 21).

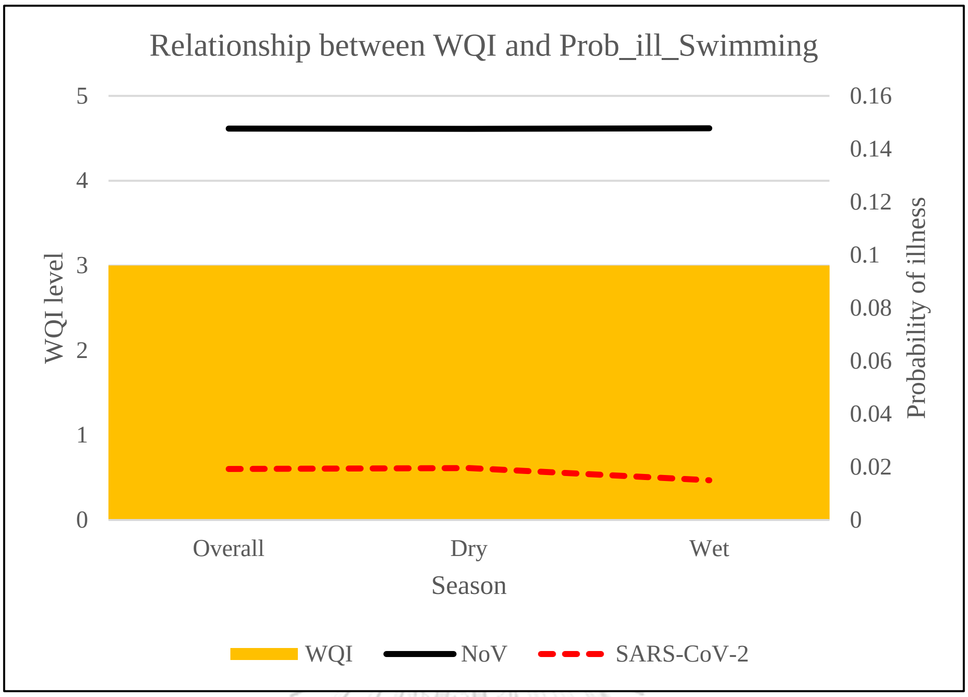


Figure 19 The seasonality relationship between WQI and the probability of illness (Swimming) of norovirus GI and SARS-CoV-2

Figure 19 shown axe X = WQI level (1 to 5), axe Y = WQI, NoV GI, SARS-CoV-2, and axe Z = probability of illness. WQI overall, dry, and wet presented “fair” water quality status between NoV GI and SARS-CoV-2 represented the probability of illness exceeds the benchmark.

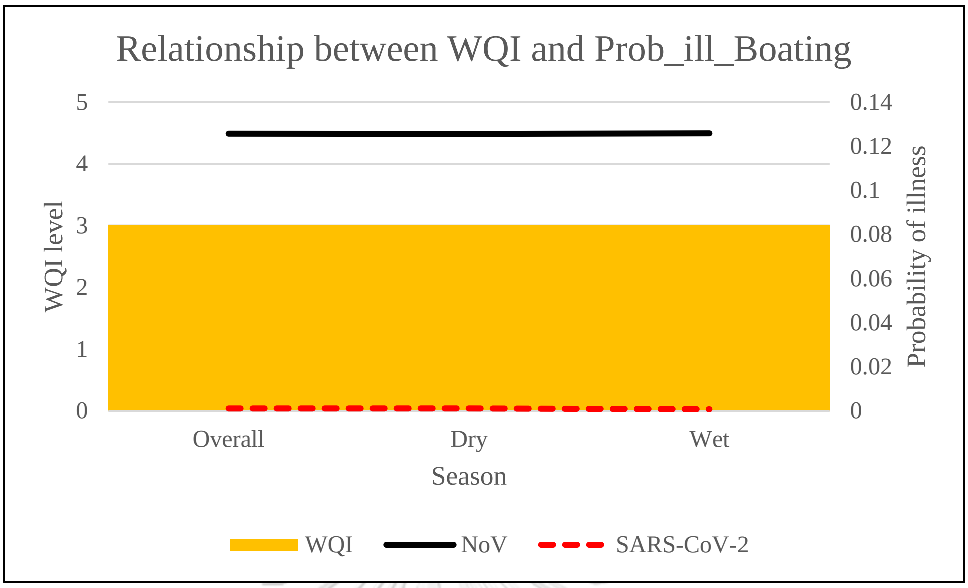


Figure 20 The seasonality relationship between WQI and the probability of illness (Boating) of norovirus GI and SARS-CoV-2

Figure 20 shown axe X = WQI level (1 to 5), axe Y = WQI, NoV GI, SARS-CoV-2, and axe Z = probability of illness. WQI overall, dry, and wet presented "fair" water quality status between NoV GI represented the probability of illness exceeds the benchmark, SARS-CoV-2 presented no exceeds the set of benchmarks.

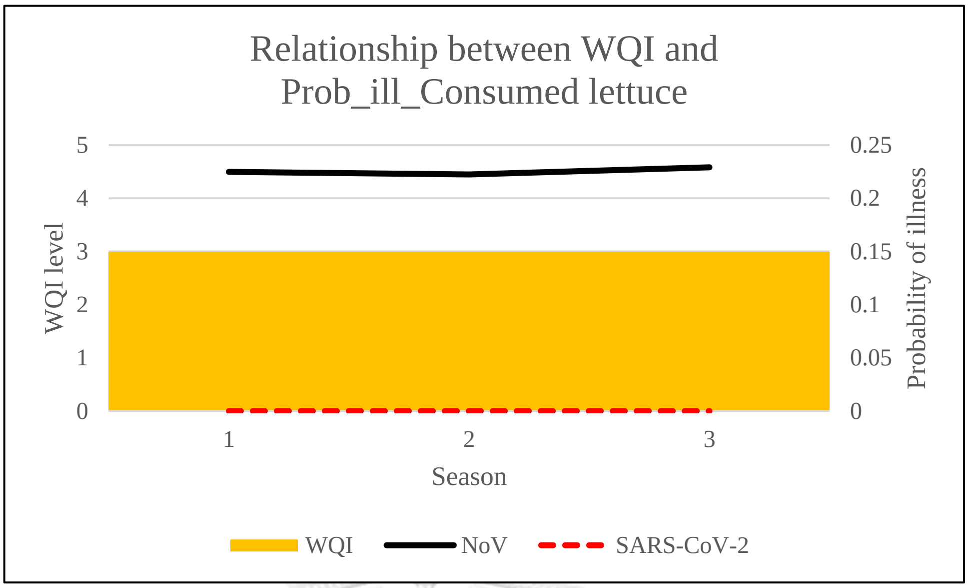


Figure 21 The seasonality relationship between WQI and the probability of illness (consumed lettuce) of norovirus GI and SARS-CoV-2

Figure 21 shown axe X = WQI level (1 to 5), axe Y = WQI, NoV GI, SARS-CoV-2, and axe Z = probability of illness. WQI overall, dry, and wet presented “fair” water quality status between NoV GI represented the probability of illness exceeds the benchmark, SARS-CoV-2 presented lowest risk of illness.

4.6.3 The relationship between the WQI each sampling site and the point estimates probability of illness on swimming, boating, and lettuce consumption of NoV GI and SARS-CoV-2

1) The relationship between the WQI and the probability of illness during swimming in the MSC from NoV GI in Figure 22 shown a moderate relationship (R=0.65) between WQI score > 50 with high probability of illness > 0.140 from NoV GI during swimming which was significantly (p – value = 0.034) associated with an increased risk of NoV GI and high WQI score, these inverse of high score meaning better quality water which the risk should be relatively low . Whereas in term of correlation level presented that at fair level of water quality index should not swimming. However, limitation of these shown the stronger of QMRA for implemented couple with the WQI. .

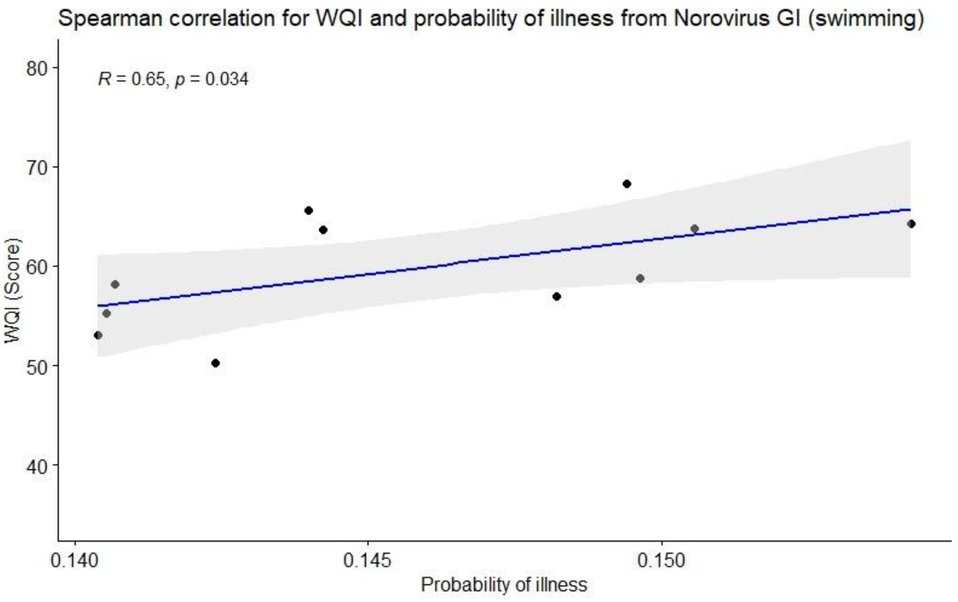


Figure 22 Spearman correlation for WQI and probability of illness from norovirus GI (Swimming)

2) The relationship between the WQI and the probability of illness during swimming in the MSC from SARS-CoV-2 in Figure 23 shown high WQI score moderate relationship (R=0.71) with high probability of illness (p – value = 0.058) that inverse from the fact of high score meaning better water quality, risk should be relatively low. Therefore, the WQI score could indicated a basic of water related activities while lower or higher score may relative with a risk from SARS-CoV-2 during swimming.

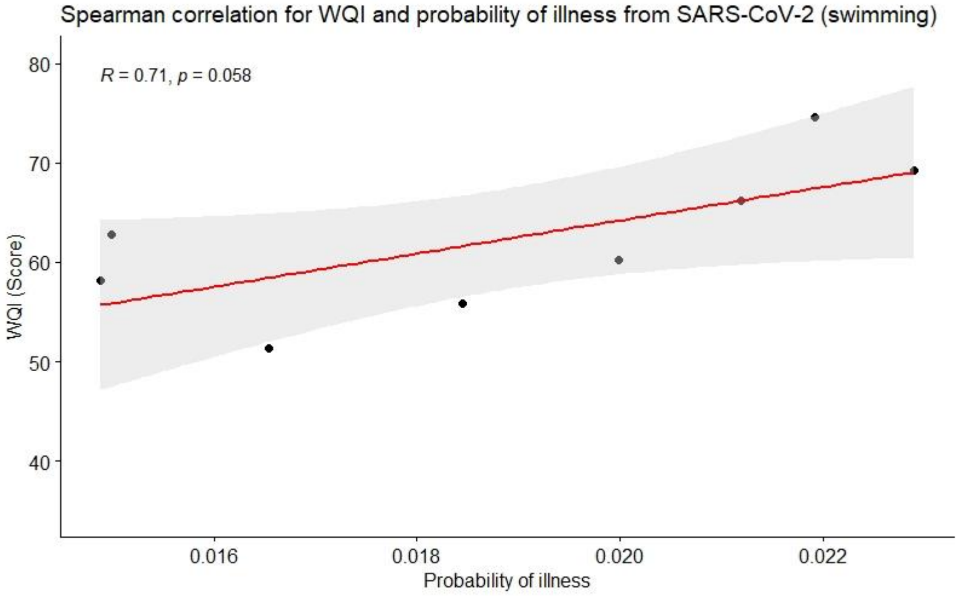


Figure 23 Spearman correlation for WQI and probability of illness from SARS-CoV-2 (Swimming)

3) The relationship between the WQI and the probability of illness during boating transportation in the MSC from NoV GI in Figure 24 shown a moderate relationship (R=0.65) between high WQI score and high probability of illness (*p* – value = 0.034) reflected to the inverse data of high score should be relatively low because WQI had high score the risk shoud be low.

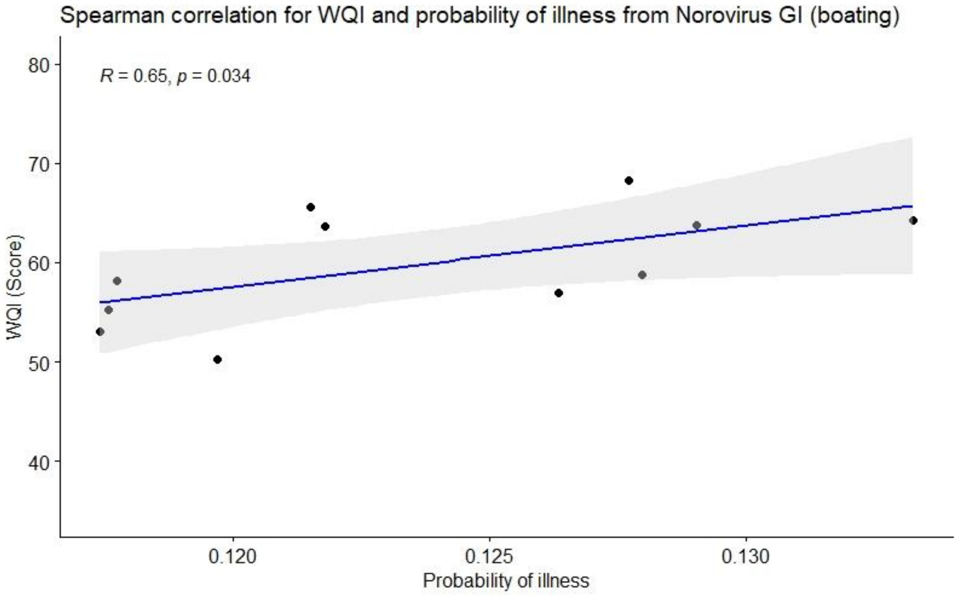


Figure 24 Spearman correlation for WQI and probability of illness from NoV GI (boating)

4) The relationship between the WQI and the probability of illness during boating transportation in the MSC from SARS-CoV-2 in Figure 25 shown trend of high WQI score increase the probability of risk which the WQI score and the probability of illness were moderate relationship (R=0.71) with high probability of illness (*p* – value = 0.058), different from the fact of the WQI high score meaning water quality is good which should be safe when transportation by boating.

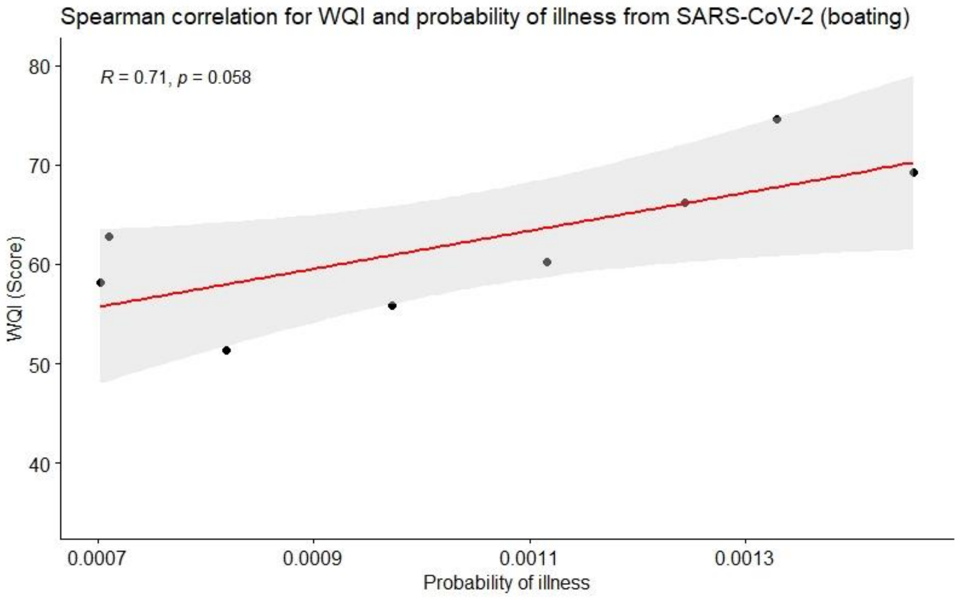


Figure 25 Spearman correlation for WQI and probability of illness from NoV GI (boating)

5) The relationship between the WQI and the probability of illness from NoV GI during consuming lettuce by planting in the MSC in Figure 26 presented the moderate relationship (R=0.68) between the WQI score and the probability of illness(*p* – value = 0.025) Otherwise, the WQI score mean about 60 score interpreted that the water quality suit to use for agriculture. This study, considered a parameter for QMRA by wash lettuce before eaten. Therefore, the WQI and the probability of illness from NoV GI inverse relationship.

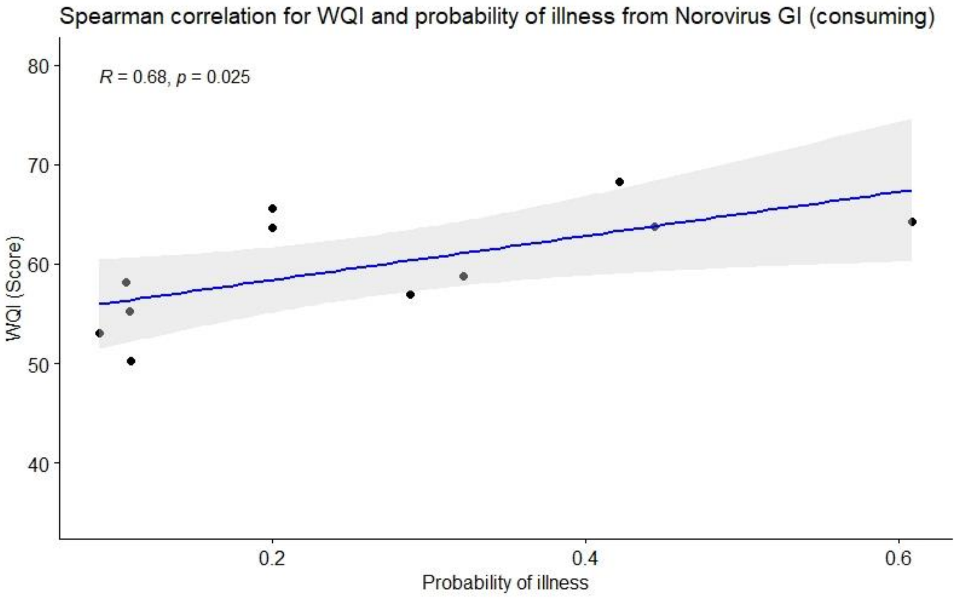


Figure 26 Spearman correlation for WQI and probability of illness from NoV GI (consuming)

6) The relationship between the WQI and the probability of illness during consuming lettuce by planting in the MSC from SARS-CoV-2 in Figure 27 cannot be conclude in term of relationship between WQI score and probability of illness during consuming lettuce by planting in the MSC from SARS-CoV-2 because the probability of illness from SARS-CoV-2 during consuming lettuce relatively lowest therefore, the WQI was suitable for agriculture while risk of SARS-CoV-2 safely level to exposed.

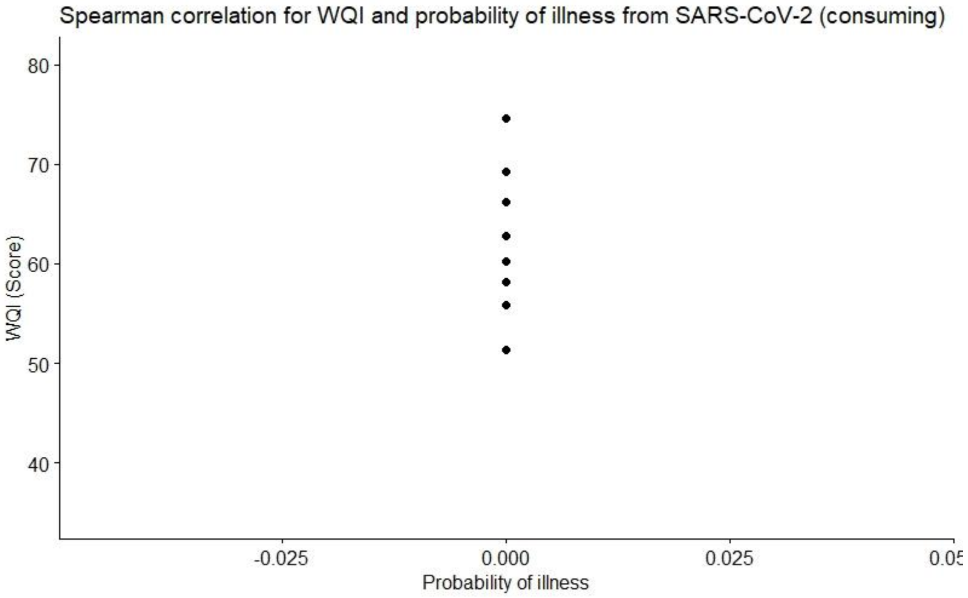


Figure 27 Spearman correlation for WQI and probability of illness from SARS-CoV-2 (consuming)

The finding point could be supporting the study hypothesizes that surface water quality status assesses by WQI indicates that surface water sources can be recreational, such as swimming or boating, the emerging and endemic virus contamination were still detected. The human health risk for waterborne pathogen contamination from water related activities is higher than benchmark, especially endemic virus.

According to NoV GI point estimation of the probability of illness in sampling sites 1 to 9 found that 77.78% (7/9) exceeded the benchmark which might be correlated with the WQI as sampling site 7 (rated as "fair") and sampling site 9 (rated as "good") associated with NoV has been lowest. Also, SARS-CoV-2 point estimation of probability of illness sites 1 to 9 found that 77.78% (7/9) not exceeded the benchmark especially sampling site 1 (rated as “poor”) and sampling site 7 (rates as “good”) of the MSC-WQI might be not associated with SARS-CoV-2 lower risk posed in surface water. Therefore, the concentration and the probability of illness in 9 sampling sites no relationship with WQI.

The overall dry season and the wet season showed a relationship with the risk of NoV GI illness that was higher than the benchmark according to the WQI, indicating that the water quality was suitable for agriculture, and transportation. However, SARS-CoV-2 illness relatively low all of overall nine-month event, dry and wet season. This implies that in certain scenarios, the WQI might not always be sufficient for identifying acceptable water-related activities. While evaluating suitable water-related activities, QMRA can be used as a further instrument for considering pathogen contamination risks into perspective as previous studies (Ishii et al., 2014) recommended that simultaneous multi-pathogen quantification could provide more dependable and comprehensive data for risk assessment than the current fecal indicator-based approach, and that microbial risk assessment and water quality monitoring are essential to ensuring safe water for drinking, recreational, and agricultural purposes.

The WQI has not align with the risk of NoV GI and SARS-CoV-2, which may be because the pathogens indicators of WQI are TCB and FCB which do not represent virus pathogens. However, (Lin & Ganesh, 2013) suggested that virological monitoring, which includes coliphages and human viruses, be included to improve the monitoring of water quality. This is because human enteric viruses may withstand changes in environmental circumstances, persist in the environment for extended periods of time, and cause diarrheal illnesses. Norovirus survived in surface water for long periods and tolerance of disinfection, this result will serve as the baseline data of the presence, detection, and risk posed in surface water-related water activities. In addition, the fate of SARS-CoV-2 is low detected, low concentration in surface water but could represent to the probability of illness during swimming recreation during the epidemic period.

Chapter 5
Conclusion and Recommendation

5.1 Conclusion

This study evaluated the suitability of water uses according to the water quality index and quantitative microbial risk assessment of noroviruses and SARS-CoV-2. The water quality in MSC was suitable for agriculture and transportation, considering the months of sampling show a “good level of water quality” might be suitable for recreation. However, novorius GI (NoV GI) and SARS-CoV-2 were detected in surface water samples during sampling period. NoV GI and SARS-CoV-2 in surface water from MSC are 41.9% (34/81) and 9.9% (8/81) respectively, indicating the potential risk associated with these viruses.

The probability of contracting an illness NoV GI due to swimming recreation, boating along the MSC, and consuming lettuce plants from irrigation water exceeds established benchmarks, while the risk of illness due to SARS-CoV-2 was not exceeds the benchmarks. Although surface water quality status assessed by WQI indicates that surface water sources can be recreational, such as swimming or boating, the emerging and endemic virus contamination was still detected, the human health risk for waterborne pathogen contamination from water-related activities is higher than the guideline. The water quality index (WQI) was used to assess the water quality, but it was found to be limited in its ability to assess the risk associated with microbial contamination. Surface water quality assessed by WQI didn’t represent the spread of SARS-CoV-2 in the surface water.

The study suggests than incorporating QMRA into the water quality evaluation can provide a more in-depth analysis, particularly when considering risks from specific pathogen contamination. It is advisable to use QMRA in conjunction with the WQI to better evaluate suitable water-related activities.

5.2 Strengths of the study

5.2.1 This is the pioneering study to assess the correlation between the Water Quality Index (WQI) and Quantitative Microbial Risk Assessment (QMRA) for both endemic and epidemic viruses in Thailand

5.2.2 The study employs the RT-qPCR method to achieve precise quantification of norovirus and SARS-CoV-2 in surface water samples.

5.3 Limitation of the study

5.3.1 The study does not estimate the health risks associated with all viruses present in the water samples.

5.3.2 It does not accurately quantify the impact of SARS-CoV-2 on gastrointestinal illnesses.

5.3.3 There is an inability to accurately measure ammonia (presumed NH_3-N refers to Ammonia as Nitrogen) levels at the water sampling sites.

5.4 Recommendation for further study

5.4.1 The study advises to use of QMRA in conjunction with the WQI to better evaluate suitable water-related activities.

5.4.2 Future studies should consider other pathogens together with a quantitative microbial risk assessment to support environmental surveillance.

5.4.3 Recommended that Water quality monitoring and microbial risk assessment are important to ensure safe water for drinking, recreational, and agricultural purposes, and simultaneous multi-pathogen quantification can provide more reliable and comprehensive information for risk assessment than the current fecal indicator-based approach.

5.5 The implications of the study

5.5.1 Provides essential baseline data for monitoring surface water quality during disease outbreaks.

The result of the study revealed information about the prevalence of endemic viruses and epidemic virus poses in surface water using norovirus and SARS-CoV-2 were pathogens representative during the COVID-19 pandemic in 2022 can be used to baseline data for surface water quality surveillance during an outbreak.

5.5.2 Facilitates the advancement of research in waterborne pathogens within higher education institutions.

This study will help to support the development of research in the pathogenic water field in higher education, especially pathogenic viruses in surface water.

5.5.3 Positions Quantitative Microbial Risk Assessment (QMRA) as a valuable tool for evaluating the risks of pathogen contamination.

In certain scenarios, the WQI might not always be sufficient for identifying acceptable water-related activities. While evaluating suitable water-related activities, QMRA can be used as a further instrument for considering pathogen contamination risks. The study recommends a comprehensive assessment of the suitability of water for specific activities together with WQI and QMRA.

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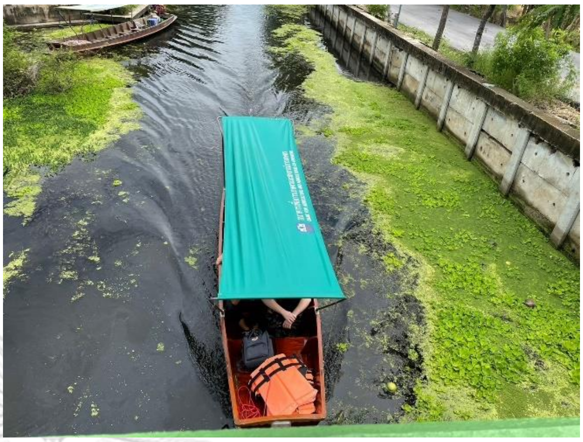
APPENDIX

Appendix 1 Sampling sites in Maha Sawat Canal

Sampling sites number	Location
1	
2	
3	

Sampling sites number	**Location**
4	
5	
6	

Sampling sites number	Location
7	
8	
9	

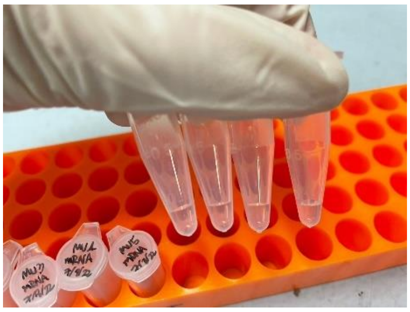
Appendix 2 The study duration and water collection time in the study area.

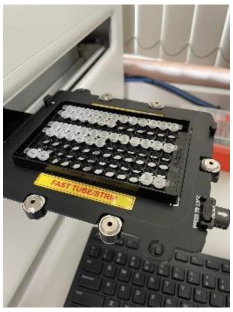
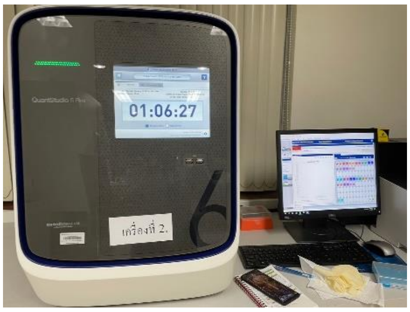
The study duration from December 2021 to August 2022 during 9 events in the dry season (December 2021 to April 2022) and the wet season (May to August 2022). and 9 water sampling sites in the Maha Sawat Canal (MSC) and the Tha Chin River of Nonthaburi, Bangkok, and Nakhon Pathom Provinces, Thailand. Regarding water sample collection, start collecting at 08.00 AM and not later than noon. Only December 2021 to January 2022 were collected later than noon PM because December was the first survey area study, and January coordinated with several research teams.

Season	**DRY**	**DRY**	**DRY**	**DRY**	**DRY**	**WET**	**WET**	**WET**	**WET**
Month	**8 DEC 2021**	**5 JAN 2022**	**5 FEB 2022**	**5 MAR 2022**	**5 APR 2022**	**5 MAY 2022**	**5 JUN 2022**	**5 JUL 2022**	**3 AUG 2022**
SS1	4.25 PM	2.05 PM	9.17 AM	8.50 AM	9.00 AM	9.50 AM	8.50 AM	9.00 AM	8.50 AM
SS2	4.05 PM	1.43 PM	9.40 AM	9.10 AM	9.16 AM	10.10 AM	9.10 AM	9.16 AM	9.10 AM
SS3	3.45 PM	1.25 PM	10.00 AM	9.30 AM	9.35 AM	10.30 AM	9.30 AM	9.35 AM	9.30 AM
SS4	3.05 PM	12.50 PM	10.33 AM	9.50 AM	10.00 AM	10.56 AM	9.50 AM	10.00 AM	9.50 AM
SS5	2.45 PM	11.20 AM	10.48 AM	10.12 AM	10.21 AM	11.16 AM	10.12 AM	10.21 AM	10.12 AM
SS6	12.30 PM	9.20 AM	11.08 AM	10.30 AM	10.40 AM	11.38 AM	10.30 AM	10.40 AM	10.30 AM
SS7	12.17 PM	9.41 AM	11.33 AM	10.53 AM	11.03 AM	12.00 PM	10.53 AM	11.03 AM	10.53 AM
SS8	11.40 AM	10.45 AM	12.10 PM	11.25 AM	11.40 AM	12.07 PM	11.25 AM	11.40 AM	11.25 AM
SS9	11.06 AM	10.05 AM	11.54 AM	11.10 AM	11.27 AM	12.40 PM	11.10 AM	11.27 AM	11.10 AM

Appendix 3 Research methodology procedure

Procedure	Illustration
Material	
Grab sampling	
Transported water sample to laboratory	

Procedure	Illustration
Virus concentration	
Genome extraction	
Quantification virus by RT-qPCR assay	

Procedure	Illustration
	 

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